3-20-00

A

Customer Number: 000959

DIVISIONAL-CONTINUATION APPLICATION TRANSMITTAL FORM UNDER RULE 1.53(b) (former Rule 1.60)

DOCKET NUMBER	ANTICIPATED CLASSIFICATION OF THIS APPLICATION:		PRIOR APPLICATION SERIAL NUMBER: 09/061,400	PRIOR APPLICATION FILING DATE: APRIL 16, 1998
MNI-056CPCN	CLASS: 530	SUBCLASS: 350	EXAMINER: L. SUN HOFFMAN	ART UNIT: 1642

ASSISTANT COMMISSIONER FOR PATENTS
BOX PATENT APPLICATION
WASHINGTON, DC 20231

CERTIFICATION UNDER 37 CFR 1.10

Date of Deposit: March 17, 2000 Mailing Label Number: EL 178 690 014 US

I hereby certify that this 37 CFR 1.53(b) request and the documents referred to as attached therein are being deposited with the United States Postal Service on the date indicated above in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and addressed to the Assistant Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

Deise K. Timas — Name of Person Mailing Paper

Deise K. Timas — Signature of Person Mailing Paper

Dear Sir:

This is a request for filing a ☒ continuation ☐ divisional application under 37 CFR 1.53(b), of pending prior Application U.S. Serial No. 09/061,400 filed on April 16, 1998, of Andrew Shyjan entitled *"Novel Multidrug Resistance-Associated Polypeptide"* which in turn is a Continuation-in-Part Application of U.S. Serial No. 08/843,459 filed on April 16, 1997, of Andrew Shyjan entitled *"Novel Multidrug Resistance-Associated Polypeptide"*.

1. ☒ Enclosed is a copy of the latest inventor signed application, including the oath or declaration as originally filed. The copy of the enclosed papers is as follows:

   ☒ 77 page(s) of Specification *(including 14 pages of Sequence Listing)*
   ☒ 11 page(s) of Claims
   ☒ 1 page(s) of Abstract
   ☒ 10 sheet(s) of Informal Drawings *(Figures 1A-G and 2A-C)*
   ☒ 5 page(s) of an *executed* Declaration, Petition and Power of attorney

   I hereby verify that the attached papers are a true copy of the prior complete application serial no. 09/061,400 filed April 16, 1998.

2. ☐ A verified statement to establish small entity status under 37 CFR 1.9 and 1.27, a copy of which is enclosed, was filed in the prior application and such status is still proper and desired (37 CFR 1.28(a)).

3. ☒ The filing fee is calculated below:

	NUMBER OF CLAIMS FILED			NUMBER EXTRA
TOTAL	* 49	MINUS	** 20	= 29
INDEP.	* 10	MINUS	*** 3	= 7
☐ MULTIPLE DEPENDENT CLAIMS				

SMALL ENTITY RATE	SMALL ENTITY FEE		OTHER THAN A SMALL ENTITY RATE	OTHER THAN A SMALL ENTITY FEE
x 9 =	$0 00	OR	x 18 =	$522.00
x 39 =	$0.00		x 78 =	$546.00
+130 =	$0.00		+ 260 =	$260.00
BASIC FEE	$0.00		BASIC FEE	$760.00
TOTAL		OR	TOTAL	$2088.00

4. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required in connection with this communication, or credit any overpayment, to Deposit Account No. 12-0080. A duplicate copy of this sheet is enclosed.

5. ☒ **THE FILING FEE IS NOT BEING PAID AT THIS TIME.**

6. ☒ Cancel in this application original claims 1-47, 49-50, 52-75 and 78-79 of the prior application and insert claims 80-114 before calculating the filing fee. (At least one original independent claim must be retained for filing purposes.)

7. ☒ A preliminary amendment is enclosed. (Claims added by this amendment have been properly numbered consecutively beginning with the number next following the highest numbered original claims in the prior application.)

8. ☐ Please abandon said prior application as of the filing date accorded this application. A duplicate copy of this transmittal is enclosed for filing in the prior application file. (May be used if signed by person authorized by §1.138 and before payment of base issue fee.)

9. ☐ New informal drawings are enclosed.

10. ☐ Priority of application serial no.______________ filed on __________________ in _____________ is claimed under 35 U.S.C. §119.
    - ☐ The certified copy has been filed in prior application serial no. ____________ filed on ________________________.
    - ☐ The certified copy will follow.

11. ☒ The prior application is assigned of record to MILLENNIUM PHARMACEUTICALS, INC.

12. ☐ A ________ month extension of time has been submitted in the parent application Serial No. __________ in order to establish copendency with the present application.

13. ☒ Also enclosed are .
    - ☒ a Preliminary Amendment; and
    - ☒ a Pre-paid Acknowledgment Postcard.

14. ☒ The power of attorney in the prior application is to Lahive & Cockfield, LLP and Millennium Pharmaceuticals, Inc..

a. ☒ The power appears in the original papers in the prior application.

b. ☐ Since the power does not appear in the original papers, a copy of the power in the prior application is enclosed.

c. ☐ A new power has been executed and is attached.

15. ☒ Address all future communications (May only be completed by applicant, or attorney or agent of record) to Elizabeth A. Hanley at **Customer Number: 000959** whose address is:

Lahive & Cockfield, LLP
28 State Street
Boston, Massachusetts 02109

16. ☒ Any requests for extensions of time necessary in a parent application for establishing copendency between this application and a parent application are hereby requested and the Commissioner is authorized to charge any fee associated with such an extension to Deposit Account No. 12-0080.

17. ☒ Pursuant to 37 CFR 1.821(e), the computer readable form of the sequence listing for this new application is to be identical with the computer readable form of application serial no. 09/061,400. Please use the computer readable form of application serial no. 09/061,400 in lieu of filing a duplicate computer readable form in this application. Pursuant to 37 CFR 1.821(f), the content of the paper copy of the sequence listing for this new application and the computer readable form of application serial no. 09/061,400 are the same.

Date: **March 17, 2000**

Elizabeth A. Hanley
Registration No. 33,505
Attorney of record

LAHIVE & COCKFIELD, LLP
28 State Street
Boston, Massachusetts 02109
Tel. (617) 227-7400

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of: Andrew Shyjan

Serial No.: Not Yet Assigned

Filed: Herewith

For: *Novel Multidrug Resistance-Associated Polypeptide*

Attorney Docket No.: MNI-056CPCN

Group Art Unit: Not Yet Assigned

Examiner: Not Yet Assigned

Assistant Commissioner for Patents
Washington, D.C. 20231

CERTIFICATION UNDER 37 CFR 1.10

Date of Deposit: March 17, 2000 Mailing Label Number: EL 178 690 014 US

I hereby certify that this 37 CFR 1.53(b) request and the documents referred to as attached therein are being deposited with the United States Postal Service on the date indicated above in an envelope as "Express Mail Post Office to Addressee" service under 37 CFR 1.10 and addressed to the Assistant Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

Deise K. Timas Deise K. Timas
Name of Person Mailing Paper Signature of Person Mailing Paper

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination of the above-identified application, please amend the application as follows:

In the Specification:

At page 1, in the first sentence following the title, after "This patent application" please insert the text -- is a continuation application of U.S. Patent Application Serial No. 09/061,400, filed on April 16, 1998 (allowed), which in turn --

At page 7, line 15, please replace "12301 Parklawn Drive, Rockville, MD 20852" with -- 10801 University Boulevard Manassas, VA 20110-2209--.

In the Claims:

Please cancel claims 1-47, 49-50, 52-75 and 78-79 without prejudice.

Please add new claims 80-114 as follows:

80. An isolated nucleic acid molecule selected from the group consisting of:

(a) an isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID No: 1;

(b) an isolated nucleic acid molecule comprising the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98409;

(c) an isolated nucleic acid molecule which is a unique fragment of the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1;

(d) at least 50% identical to the nucleotide sequence of SEQ ID No: 1;

(e) an isolated nucleic acid molecule that hybridizes under stringent conditions to the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1;

(f) an isolated nucleic acid molecule which is a degenerate sequence variant of the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1;

(g) an isolated nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID No: 2;

(h) an isolated nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence which is at least 75% identical to the polypeptide having the amino acid sequence of SEQ ID No: 2; and

(i) an isolated nucleic acid molecules which is complementary to the nucleic acid molecule in any of subparts (a), (b) or (e).

81. An oligonucleotide selected from the group consisting of:

(a) an oligonucleotide that hybridizes to a unique fragment of the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1;

(b) an oligonucleotide that hybridizes to a unique fragment of the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1 under stringent conditions;

(c) an oligonucleotide that hybridizes to a unique fragment of the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1 under intracellular conditions;

(d) an oligonucleotide as in subpart (a) comprising at least one modification in a nucleotide base, backbone sugar, phosphate or sugar-phosphate linkage;

(e) an oligonucleotide as in subpart (a) comprising a peptide nucleic acid backbone;

(f) an oligonucleotide as in subpart (a) which is detectably labeled;

(g) an oligonucleotide as in subpart (a) which is biotinylated, radiolabeled or fluorophore-conjugated;

(h) an oligonucleotide as in subpart (a) wherein said unique fragment is at least 9 nucleotides in length;

(i) an oligonucleotide as in subpart (a) wherein said unique fragment is at least 15 nucleotides in length;

(j) an oligonucleotide as in subpart (a) wherein said unique fragment is at least 21 nucleotides in length;

(k) an oligonucleotide as in subpart (a) wherein said unique fragment is a locus comprising a 5' untranslated sequence, transcription initiation site, coding sequence, intron-exon boundary, polyadenylation site, or 3' untranslated sequence in the nucleic acid of SEQ ID No: 1; and

(l) an oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID Nos: 4, 5, 6, 7 and 8.

82. An antisense vector comprising the oligonucleotide of claim 81.

83. An antisense pharmaceutical composition comprising the oligonucleotide of claim 81 or a vector of comprising said oligonucleotide, dispersed in a pharmaceutically acceptable vehicle.

84. An isolated MRP-β polypeptide selected from the group consisting of:

(a) a polypeptide comprising the amino acid sequence of SEQ ID No: 2;

(b) a polypeptide comprising an amino acid sequence sharing at least 75% sequence identity with the amino acid sequence of SEQ ID No: 2;

(c) a polypeptide which is an epitope unique to the MRP-β polypeptide having the amino acid sequence of SEQ ID No: 2; and

(d) the polypeptide as set forth in subpart (c), where said epitope is displayed by a cell expressing an MRP-β gene.

85. An antibody that binds selectively to the polypeptide of claim 84, or an antigen-binding fragment thereof.

86. A fusion polypeptide selected from the group consisting of:

(a) a fusion polypeptide comprising an antigen-binding fragment of claim 85.

(b) a fusion polypeptide as set forth in subpart (a) further comprising a cytotoxic polypeptide, such that said fusion polypeptide stimulates cytolysis of a cell expressing an MRP-β gene; and

(c) a fusion polypeptide as set forth in subpart (a) further comprising a chemoattractant, such that said fusion polypeptide stimulates destruction of a cell expressing an MRP-β gene by macrophages, killer T cells or cytotoxic T cells.

87. An expression vector comprising a nucleic acid molecule encoding the polypeptide of claim 84.

88. A cell selected from the group consisting of:

(a) a cell transfected with the expression vector of claim 87;

(b) a cell transfected with the expression vector of claim 87, wherein said cell is immortalized under cell culture conditions;

(c) a cell as in subpart (b), wherein said cell is of human origin;

(d) a cell as in subpart (b), wherein said cell is a unicellular organism;

(e) a cell as in subpart (d), wherein said cell is yeast cell; and

(f) a cell as in subpart (a), wherein said cell is a non-human mammalian embryonic blastocyst cell.

89. A non-human mammal produced by intrauterine implantation of a blastocyst comprising a cell transfected with an expression vector comprising a nucleic acid molecule encoding the polypeptide of claim 84, wherein said cell is a non-human mammalian embryonic blastocyst cell.

90. A progeny of the mammal of claim 89, said progeny characterized by germline integration of said nucleic acid encoding said polypeptide.

91. A null vector comprising nucleic acid encoding a non-expressible variant of a polypeptide having an amino acid sequence sharing at least 75% sequence identity with the amino acid sequence of SEQ ID No: 2

92. A cell transfected with the null vector of claim 91.

93. The cell of claim 92, wherein said cell is a non-human mammalian embryonic blastocyst cell.

94. A non-human mammal produced by intrauterine implantation of a blastocyst comprising the cell of claim 93.

95. A progeny of the mammal of claim 94, said progeny characterized by germline integration of said nucleic acid molecule.

96. A method of detecting expression of an MRP-β gene, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring cells expressing an MRP-β gene encoding a polypeptide comprising the amino acid sequence of SEQ ID No: 2;

(b) releasing RNA from said cellular tissue;

(c) combining, under hybridization conditions, said released RNA with an oligonucleotide that hybridizes to the complement of SEQ ID No: 1 or a unique fragment thereof; and

(d) assaying said released RNA for formation of a hybrid comprising said oligonucleotide, formation of which indicates that cells of said tissue express said MRP-β gene.

97. The method of claim 48 or 96, wherein said cellular tissue is suspected of comprising transformed cells.

98. The method of claim 48, 51 or 96, wherein said oligonucleotide comprises a peptide nucleic acid backbone.

99. A method of characterizing drug-resistant phenotype of a transformed cell of mammalian origin, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring transformed cells;

(b) contacting said tissue with an antibody of claim 85, under conditions such that, if cells of said tissue display said an epitope selectively bound by said antibody, an antibody-epitope complex forms; and,

(c) assaying said tissue for the presence of said complex, formation of which indicates presence of transformed cells having a drug-resistant phenotype in said mammal.

100. The method of claim 51 or 99 wherein said cellular tissue is selected form the group consisting of:

(a) cellular tissue which is of mammary, respiratory tract, urogenital tract, endocrine system or immune system origin;

(b) cellular tissue which is of mammary origin and comprises a breast biopsy sample;

(c) cellular tissue which is of respiratory tract origin and comprises a bronchoalveolar lavage sample;

(d) cellular tissue which is of urogenital tract origin and comprises an ovarian, uterine or cervical biopsy sample;

(e) cellular tissue which is of urogenital tract origin and comprises a prostate or testicular biopsy sample;

(f) cellular tissue which is of endocrine system origin and comprises a pancreatic biopsy sample; and

(g) cellular tissue which is of immune system origin and comprises a spleen, bone marrow or lymph node biopsy sample.

101. A method of mitigating aberrant expression of an MRP-β gene, comprising administering an antisense pharmaceutical composition of claim 83 to a mammal suffering from effects of said aberrant expression, under conditions sufficient to attenuate a phenotype associated therewith.

102. A method of mitigating aberrant activity of an MRP-β gene, comprising administering an antisense pharmaceutical composition of claim 83 to a mammal suffering from effects of said aberrant activity, under conditions sufficient to attenuate a phenotype associated therewith.

103. A method of improving effectiveness of chemotherapy for a mammal afflicted with a multidrug-resistant tumor, comprising administering a chemotherapeutic drug to said mammal; and coadministering an antisense pharmaceutical composition of claim 83, such that said antisense pharmaceutical composition mitigates resistance of said tumor to said chemotherapeutic drug.

104. A method of treating a mammal suffering from aberrant expression of an MRP-β gene or a mammal suffering from aberrant activity of an MRP-β, comprising administering a

fusion polypeptide of claim 86 to said mammal, in an amount effective for destroying cells aberrantly displaying an epitope unique to an MRP-β polypeptide.

105. A method of treating a mammal afflicted with a multidrug-resistant tumor, comprising the step of administering a fusion polypeptide of claim 86 to said mammal, in an amount effective for destroying tumor cells displaying an epitope unique to an MRP-β polypeptide.

106. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell transfected with a vector comprising a nucleic acid molecule encoding the polypeptide of claim 84 with a candidate modulator of MRP-β;

(b) assaying the level of MRP-β gene expression or MRP-β polypeptide expression in said cell, wherein a detectable fluctuation in said level indicates that said candidate is an MRP-β modulator.

107. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell transfected with a vector comprising a nucleic acid molecule encoding the polypeptide of claim 84 with a substrate transported by MRP-β;

(b) contacting said cell with a candidate modulator of MRP-β;

(c) assaying the amount of said substrate exported by said cell, wherein a detectable fluctuation in said amount indicates that said candidate is an MRP-β modulator.

108. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell transfected with a vector comprising a nucleic acid molecule encoding the polypeptide of claim 84 with a cytotoxin exported or sequestered by MRP-β;

(b) contacting said cell with a candidate modulator of MRP-β;

(c) assaying survival of said cell, a detectable fluctuation in which indicates that said candidate is an MRP-β modulator.

109. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell transfected with a vector comprising a nucleic acid molecule encoding the polypeptide of claim 84 with a cytotoxin exported by MRP-β;

(b) contacting said with a candidate modulator of MRP-β;

(c) assaying efflux of said cytotoxin from said cell, a fluctuation in which indicates that said candidate is an MRP-β modulator.

110. An MRP-β modulator identified by the method of claim 106, 107, 108 or 109.

111. An MRP-β modulator of claim 110, wherein said modulator is an inhibitor or a small molecule.

112. A multidrug-resistance attenuating pharmaceutical composition comprising an MRP-β modulator dispersed in a pharmaceutically acceptable vehicle.

113. A method of improving effectiveness of chemotherapy for a mammal afflicted with a multidrug-resistant tumor, comprising the steps of:

(a) administering a chemotherapeutic drug to said mammal; and,

(b) coadministering a pharmaceutical composition of claim 112,

such that said composition mitigates resistance of said tumor to said chemotherapeutic drug.

114. The method of claim 113 wherein said tumor is of mammary, respiratory tract, urogenital tract, endocrine system of immune system origin.

REMARKS

The specification has been amended to recite the date of deposit and the current address of the ATCC depository.

Claims 1-79 were previously pending in the present application. Claims 1-47, 49-50, 52-75 and 78-79 have been cancelled without prejudice to further prosecution in this or another

application. New claims 80-114 have been added. Accordingly, claims 48, 51, 76-77 and 80-114 are currently pending in this application. For the Examiner's convenience, the claims that will be pending after entry of the instant amendment are set forth in APPENDIX A.

The foregoing claim cancellations were made solely to expedite prosecution. Applicant reserves the right to pursue the same or similar subject matter as encompassed by the amended and/or cancelled claims herein or as originally filed in this or a separate application(s).

No new matter has been added to the application.

CONCLUSION

If a telephone conversation with Applicants' Attorney would expedite prosecution of the above-identified application, the Examiner is urged to call the undersigned at (617) 227-7400.

Date: March 17, 2000

LAHIVE & COCKFIELD, LLP
Attorneys at Law

By [signature]

Elizabeth A. Hanley
Reg. No. 33,505
28 State Street
Boston, MA 02109
(617) 227-7400
(617) 742-4214

APPENDIX A

48. A method of detecting a mutation in an MRP-β gene, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring a variant MRP-β gene, the sequence of which differs from SEQ ID No: 1 by at least one nucleotide substitution, insertion or deletion;

(b) releasing nucleic acids from said cellular tissue;

(c) combining, under hybridization conditions, said released nucleic acids with an oligonucleotide complementary to SEQ ID No: 1 or to a unique fragment thereof; and

(d) assaying said released nucleic acids for formation of a hybrid comprising said oligonucleotide, formation of which indicates that said mammal harbors at least one wild-type MRP-β gene allele, the sequence of which comprises SEQ ID No: 1.

49. A method of detecting expression of an MRP-β gene, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring cells expressing an MRP-β gene encoding a polypeptide of claim 25;

(b) releasing RNA from said cellular tissue;

(c) combining, under hybridization conditions, said released RNA with an oligonucleotide that hybridizes to the complement of SEQ ID No: 1 or a unique fragment thereof; and

(d) assaying said released RNA for formation of a hybrid comprising said oligonucleotide, formation of which indicates that cells of said tissue express said MRP-β gene.

51. A method of characterizing multidrug-resistant phenotype of a transformed cell of mammalian origin, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring transformed cells;

(b) releasing RNA from said cellular tissue;

(c) combining, under hybridization conditions, said released RNA with an oligonucleotide that hybridizes to the complement of SEQ ID No: 1 or a unique fragment thereof; and

(d) assaying said released RNA for formation of a hybrid comprising said oligonucleotide, formation of which indicates presence of transformed cells having a multidrug-resistance phenotype.

76. A method of mitigating aberrant expression of an MRP-β gene, comprising the step of administering an MRP-β modulator to a mammal suffering from effects of said aberrant expression, under conditions sufficient to attenuate a phenotype associated therewith.

77. A method of treating a mammal suffering from aberrant activity of an MRP-β polypeptide, comprising the step of administering an MRP-β modulator to a mammal suffering from effects of said aberrant activity, under conditions sufficient to attentuate a phenotype associated therewith.

80. An isolated nucleic acid molecule selected from the group consisting of:

(a) an isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID No: 1;

(b) an isolated nucleic acid molecule comprising the nucleotide sequence of the DNA insert of the plasmid deposited with ATCC as Accession Number 98409;

(c) an isolated nucleic acid molecule which is a unique fragment of the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1;

(d) at least 50% identical to the nucleotide sequence of SEQ ID No: 1;

(e) an isolated nucleic acid molecule that hybridizes under stringent conditions to the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1;

(f) an isolated nucleic acid molecule which is a degenerate sequence variant of the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1;

(g) an isolated nucleic acid molecule which encodes a polypeptide comprising the amino acid sequence of SEQ ID No: 2;

(h) an isolated nucleic acid molecule which encodes a polypeptide comprising an amino acid sequence which is at least 75% identical to the polypeptide having the amino acid sequence of SEQ ID No: 2; and

(i) an isolated nucleic acid molecules which is complementary to the nucleic acid molecule in any of subparts (a), (b) or (e).

81. An oligonucleotide selected from the group consisting of:

(a) an oligonucleotide that hybridizes to a unique fragment of the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1;

(b) an oligonucleotide that hybridizes to a unique fragment of the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1 under stringent conditions;

(c) an oligonucleotide that hybridizes to a unique fragment of the nucleic acid molecule having the nucleotide sequence of SEQ ID No: 1 under intracellular conditions;

(d) an oligonucleotide as in subpart (a) comprising at least one modification in a nucleotide base, backbone sugar, phosphate or sugar-phosphate linkage;

(e) an oligonucleotide as in subpart (a) comprising a peptide nucleic acid backbone;

(f) an oligonucleotide as in subpart (a) which is detectably labeled;

(g) an oligonucleotide as in subpart (a) which is biotinylated, radiolabeled or fluorophore-conjugated;

(h) an oligonucleotide as in subpart (a) wherein said unique fragment is at least 9 nucleotides in length;

(i) an oligonucleotide as in subpart (a) wherein said unique fragment is at least 15 nucleotides in length;

(j) an oligonucleotide as in subpart (a) wherein said unique fragment is at least 21 nucleotides in length;

(k) an oligonucleotide as in subpart (a) wherein said unique fragment is a locus comprising a 5' untranslated sequence, transcription initiation site, coding sequence, intron-exon boundary, polyadenylation site, or 3' untranslated sequence in the nucleic acid of SEQ ID No: 1; and

(l) an oligonucleotide having a nucleotide sequence selected from the group consisting of SEQ ID Nos: 4, 5, 6, 7 and 8.

82. An antisense vector comprising the oligonucleotide of claim 81.

83. An antisense pharmaceutical composition comprising the oligonucleotide of claim 81 or a vector of comprising said oligonucleotide, dispersed in a pharmaceutically acceptable vehicle.

84. An isolated MRP-β polypeptide selected from the group consisting of:

(a) a polypeptide comprising the amino acid sequence of SEQ ID No: 2;

(b) a polypeptide comprising an amino acid sequence sharing at least 75% sequence identity with the amino acid sequence of SEQ ID No: 2;

(c) a polypeptide which is an epitope unique to the MRP-β polypeptide having the amino acid sequence of SEQ ID No: 2; and

(d) the polypeptide as set forth in subpart (c), where said epitope is displayed by a cell expressing an MRP-β gene.

85. An antibody that binds selectively to the polypeptide of claim 84, or an antigen-binding fragment thereof.

86. A fusion polypeptide selected from the group consisting of:

(a) a fusion polypeptide comprising an antigen-binding fragment of claim 85.

(b) a fusion polypeptide as set forth in subpart (a) further comprising a cytotoxic polypeptide, such that said fusion polypeptide stimulates cytolysis of a cell expressing an MRP-β gene; and

(c) a fusion polypeptide as set forth in subpart (a) further comprising a chemoattractant, such that said fusion polypeptide stimulates destruction of a cell expressing an MRP-β gene by macrophages, killer T cells or cytotoxic T cells.

87. An expression vector comprising a nucleic acid molecule encoding the polypeptide of claim 84.

88. A cell selected from the group conssiting of:

(a) a cell transfected with the expression vector of claim 87;

(b) a cell transfected with the expression vector of claim 87, wherein said cell is immortalized under cell culture conditions;

(c) a cell as in subpart (b), wherein said cell is of human origin;

(d) a cell as in subpart (b), wherein said cell is a unicellular organism;

(e) a cell as in subpart (d), wherein said cell is yeast cell; and

(f) a cell as in subpart (a), wherein said cell is a non-human mammalian embryonic blastocyst cell.

89. A non-human mammal produced by intrauterine implantation of a blastocyst comprising a cell transfected with an expression vector comprising a nucleic acid molecule encoding the polypeptide of claim 84, wherein said cell is a non-human mammalian embryonic blastocyst cell.

90. A progeny of the mammal of claim 89, said progeny characterized by germline integration of said nucleic acid encoding said polypeptide.

91. A null vector comprising nucleic acid encoding a non-expressible variant of a polypeptide having an amino acid sequence sharing at least 75% sequence identity with the amino acid sequence of SEQ ID No: 2

92. A cell transfected with the null vector of claim 91.

93. The cell of claim 92, wherein said cell is a non-human mammalian embryonic blastocyst cell.

94. A non-human mammal produced by intrauterine implantation of a blastocyst comprising the cell of claim 93.

95. A progeny of the mammal of claim 94, said progeny characterized by germline integration of said nucleic acid molecule.

96. A method of detecting expression of an MRP-β gene, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring cells expressing an MRP-β gene encoding a polypeptide comprising the amino acid sequence of SEQ ID No: 2;

(b) releasing RNA from said cellular tissue;

(c) combining, under hybridization conditions, said released RNA with an oligonucleotide that hybridizes to the complement of SEQ ID No: 1 or a unique fragment thereof; and

(d) assaying said released RNA for formation of a hybrid comprising said oligonucleotide, formation of which indicates that cells of said tissue express said MRP-β gene.

97. The method of claim 48 or 96, wherein said cellular tissue is suspected of comprising transformed cells.

98. The method of claim 48, 51 or 96, wherein said oligonucleotide comprises a peptide nucleic acid backbone.

99. A method of characterizing drug-resistant phenotype of a transformed cell of mammalian origin, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring transformed cells;

(b) contacting said tissue with an antibody of claim 85, under conditions such that, if cells of said tissue display said an epitope selectively bound by said antibody, an antibody-epitope complex forms; and,

(c) assaying said tissue for the presence of said complex, formation of which indicates presence of transformed cells having a drug-resistant phenotype in said mammal.

100. The method of claim 51 or 99 wherein said cellular tissue is selected form the group consisting of:

(a) cellular tissue which is of mammary, respiratory tract, urogenital tract, endocrine system or immune system origin;

(b) cellular tissue which is of mammary origin and comprises a breast biopsy sample;

(c) cellular tissue which is of respiratory tract origin and comprises a bronchoalveolar lavage sample;

(d) cellular tissue which is of urogenital tract origin and comprises an ovarian, uterine or cervical biopsy sample;

(e) cellular tissue which is of urogenital tract origin and comprises a prostate or testicular biopsy sample;

(f) cellular tissue which is of endocrine system origin and comprises a pancreatic biopsy sample; and

(g) cellular tissue which is of immune system origin and comprises a spleen, bone marrow or lymph node biopsy sample.

101. A method of mitigating aberrant expression of an MRP-β gene, comprising administering an antisense pharmaceutical composition of claim 83 to a mammal suffering from effects of said aberrant expression, under conditions sufficient to attenuate a phenotype associated therewith.

102. A method of mitigating aberrant activity of an MRP-β gene, comprising administering an antisense pharmaceutical composition of claim 83 to a mammal suffering from effects of said aberrant activity, under conditions sufficient to attenuate a phenotype associated therewith.

103. A method of improving effectiveness of chemotherapy for a mammal afflicted with a multidrug-resistant tumor, comprising administering a chemotherapeutic drug to said

mammal; and coadministering an antisense pharmaceutical composition of claim 83, such that said antisense pharmaceutical composition mitigates resistance of said tumor to said chemotherapeutic drug.

104. A method of treating a mammal suffering from aberrant expression of an MRP-β gene or a mammal suffering from aberrant activity of an MRP-β, comprising administering a fusion polypeptide of claim 86 to said mammal, in an amount effective for destroying cells aberrantly displaying an epitope unique to an MRP-β polypeptide.

105. A method of treating a mammal afflicted with a multidrug-resistant tumor, comprising the step of administering a fusion polypeptide of claim 86 to said mammal, in an amount effective for destroying tumor cells displaying an epitope unique to an MRP-β polypeptide.

106. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell transfected with a vector comprising a nucleic acid molecule encoding the polypeptide of claim 84 with a candidate modulator of MRP-β;

(b) assaying the level of MRP-β gene expression or MRP-β polypeptide expression in said cell, wherein a detectable fluctuation in said level indicates that said candidate is an MRP-β modulator.

107. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell transfected with a vector comprising a nucleic acid molecule encoding the polypeptide of claim 84 with a substrate transported by MRP-β;

(b) contacting said cell with a candidate modulator of MRP-β;

(c) assaying the amount of said substrate exported by said cell, wherein a detectable fluctuation in said amount indicates that said candidate is an MRP-β modulator.

108. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell transfected with a vector comprising a nucleic acid molecule encoding the polypeptide of claim 84 with a cytotoxin exported or sequestered by MRP-β;

(b) contacting said cell with a candidate modulator of MRP-β;

(c) assaying survival of said cell, a detectable fluctuation in which indicates that said candidate is an MRP-β modulator.

109. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell transfected with a vector comprising a nucleic acid molecule encoding the polypeptide of claim 84 with a cytotoxin exported by MRP-β;

(b) contacting said with a candidate modulator of MRP-β;

(c) assaying efflux of said cytotoxin from said cell, a fluctuation in which indicates that said candidate is an MRP-β modulator.

110. An MRP-β modulator identified by the method of claim 106, 107, 108 or 109.

111. An MRP-β modulator of claim 110, wherein said modulator is an inhibitor or a small molecule.

112. A multidrug-resistance attenuating pharmaceutical composition comprising an MRP-β modulator dispersed in a pharmaceutically acceptable vehicle.

113. A method of improving effectiveness of chemotherapy for a mammal afflicted with a multidrug-resistant tumor, comprising the steps of:

(a) administering a chemotherapeutic drug to said mammal; and,

(b) coadministering a pharmaceutical composition of claim 112,

such that said composition mitigates resistance of said tumor to said chemotherapeutic drug.

114. The method of claim 113 wherein said tumor is of mammary, respiratory tract, urogenital tract, endocrine system of immune system origin.

Novel Multidrug Resistance-Associated Polypeptide

This patent application is a continuation-in-part of U.S. Serial Number 08/843,459, filed April 16, 1997, the disclosure of which is incorporated herein by reference.

Field of the Invention

The present invention relates generally to cancer chemotherapy. The invention relates more specifically to compositions and methods for improving the effectiveness of a chemotherapeutic regimen to eradicate multidrug-resistant transformed cells from the body of a mammal, preferably from the body of a human. In this regard, the invention capitalizes on the discovery of a novel multidrug-resistance associated polypeptide (MRP), herein designated MRP-β. The invention further relates to drug discovery, especially to the design of novel chemotherapeutic drugs that are cytotoxic to cells expressing MRP-β .

Background of the Invention

Cancer chemotherapy involves the administration of one or more cytotoxic or cytostatic drugs to a cancer sufferer. The goal of chemotherapy is to eradicate a substantially clonal population (colony) of transformed cells from the body of the individual, or to suppress or to attenuate growth of the colony, which is most commonly referred to as a tumor. Tumors may occur in solid or liquid form, the latter comprising a cell suspension in blood or another body fluid. A secondary goal of chemotherapy is stabilization (clinical management) of the afflicted individual's health status. Although the tumor may initially respond to chemotherapy by, e.g., stabilizing or reducing its growth rate, in many instances the initial chemotherapeutic treatment regimen becomes less effective or ceases to impede tumor growth. Conventional treatment regimes

endorse the use of additional or substitute chemotherapeutic drugs, including drug combinations, in an effort to regain control over tumor growth. However, it is well known that transformed cells in a tumor may acquire resistance to a broad spectrum of chemotherapeutic drugs, including drugs to which the tumor has not hitherto been exposed during treatment. This acquisition of a multidrug-resistant (or multidrug-resistance) phenotype significantly constrains the chemotherapeutic choices available to the clinician, and significantly worsens prognosis for the afflicted individual. Acquisition of multidrug resistance is particularly problematic in carcinomas originating in secretory epithelia, including lung, gastrointestinal tract, mammary, reproductive tract, endocrine and neuroendocrine epithelia.

Tumor cell transformation is the process by which a cell escapes normal control mechanisms governing the cell's tissue-specific phenotype and differentiation state. Thus, transformation often involves "dedifferentiation," which is defined as an inappropriate return to a less committed or less tissue-specific phenotype. Alternatively, transformation involves incomplete or arrested differentiation of cells normally responsible for replenishing cells lost to normal tissue turnover. Transformed cells of epithelial origin produce tumors that are carcinoma cell colonies (carcinomas). When in a gland-like configuration or derived from secretory tissue, such epithelium-derived tumors are referred to as adenocarcinomas. In contrast, transformed cells of mesenchymal origin produce tumors that are sarcoma cell colonies (sarcomas). Transformed cells of the hematopoietic lineage produce leukemias, lymphomas or lymphosarcomas, each of which often occur as cell suspension tumors. In contrast, the primary tumor growth of a carcinoma or sarcoma usually remains near the site of initial cell transformation. However, secondary foci (metastases) of tumor growth can arise at other sites, which can be far removed from the primary tumor growth site. The presence and/or abundance of metastases indicates the degree to which transformed cells have strayed from their normal tissue-specific phenotype and/or acquired invasive properties.

Phenotypically, cell transformation involves the display of altered or abnormal structural (e.g. antigenic) and functional cellular properties. These altered properties provide the transformed cell with a survival or growth advantage over neighboring, non-transformed cells in its tissue of origin. The advantage may arise from acquisition of autocrine growth regulation, abnormal activation of genes controlling or regulating the cell division cycle, abnormal suppression of genes needed for normal exit from or arrest of the cell division cycle, or other changes affecting cell growth and/or survival. Over time, divisions of the transformed cell produce a colony (tumor) of daughter cells each having the phenotypic advantage gained by the original transformed cell. The imposition of chemotherapy subjects the tumor to selection pressure, in effect encouraging further phenotypic change by which tumor cells may escape the cytotoxic effects of a chemotherapeutic drug. Thus, the structural and functional properties of transformed cells in a tumor can fluctuate over time and over the course of chemotherapeutic treatment.

A significant survival advantage is associated with the acquisition of a multidrug-resistance phenotype, which arises from expression of a cellular gene encoding a protein that removes diverse chemotherapeutic drugs or drug metabolites from the intracellular milieu. Drug export diminishes cytotoxic effect, thereby protecting the transformed cell from otherwise lethal chemotherapeutic drugs or drug concentrations. To date, two genes encoding multidrug-resistance export proteins have been identified in the human genome. The first of these, MDR1, encodes P-glycoprotein, a 170 kDa multispanning transmembrane protein belonging the ATP Binding Cassette (ABC) Transporter protein superfamily. Lautier et al. (1996), 52 Biochem. Pharmacol. 967-977. Superfamily members are multispanning transmembrane proteins that transport substances into or out of the intracellular environment in an energy-dependent manner. Higgins (1992), 8 Ann. Rev. Cell Biol. 67-113, provides a general overview of the properties and natural occurrence of superfamily member proteins. ABC transporters have been identified for a large variety

of structurally diverse transported substrates, including sugars, peptides, inorganic ions, amino acids, polysaccharides and proteins. Individual transporter proteins appear to function unidirectionally, i.e., to carry out either export or import of intracellular substances. Thus, P-glycoprotein functions by exporting chemotherapeutic drugs which, although structurally heterogenous, appear to share hydrophobic properties. P-glycoprotein overexpression correlates with the presence of a multidrug-resistance phenotype in diverse tumor cell isolates and tumorigenic cell lines. Significant effort has been invested in the development of agents to block or attenuate P-glycoprotein mediated drug export. Such agents are referred to commonly as "chemosensitizers" or "MDR reversal agents," and are disclosed in Hait et al. (1992), U.S. Patent 5,104,858; Sunkara et al. (1993), U.S. Patent 5,182,293; Sunkara et al. (1993), U.S. Patent 5,190,957; Ramu et al. (1993), U.S. Patent 5,190,946; Powell et al. (1995), U.S. Patent 5,387,685; Piwnica-Worms (1995), U.S. Patent 5,403,574; Sarkadi et al. (1995), PCT Publication WO 95/31474; Sunkara et al. (1996), U.S. Patent 5,523,304; Zelle et al. (1996), U.S. Patent 5,543,423; Engel et al. (1996), U.S. Patent 5,556,856; Powell et al. (1996), U.S. Patent 5,550,149 and Powell et al. (1996), U.S. Patent 5,561,141. However, P-glycoprotein overexpression does not account for all instances of the acquisition of a multidrug-resistance phenotype. Lautier et al. (1996), 52 Biochem. Pharmacol. 967-977.

A second multidrug-resistance gene identified to date in the human genome encodes multidrug-resistance associated protein (MRP), a 190 kDa multispanning transmembrane protein also belonging to the ABC Transporter protein superfamily. MRP is described in Deeley et al. (1996), U.S. Patent 5,489,519, the teachings of which are incorporated by reference herein. MRP shares only 15% sequence identity with P-glycoprotein at the amino acid level. In addition, MRP differs from P-glycoprotein in its ability to expel specific types of chemotherapeutic drugs from the intracellular milieu. These differences are thought to arise from differences in the drug expulsion mechanism of the two proteins: MRP appears to act on a glutathione-derivatized drug metabolite,

whereas P-glycoprotein appears to act on an underivatized drug. Lautier et al. (1996), 52 Biochem. Pharmacol. 967-977. Significantly, agents that block or interfere with P-glycoprotein function appear to have little crossreactivity with MRP. Thus, significant effort is being invested in the development of substances (MDR reversal agents) that block or inhibit MRP function.

Overexpression of either P-glycoprotein or MRP can endow a transformed cell with a multidrug-resistance phenotype; thus, empirical testing is required to determine whether a particular reversal agent will be effective for interfering with a tumor's multidrug resistance phenotype. Currently, it is unclear whether MRP and/or P-glycoprotein expression accounts for all occurrences of the multidrug-resistance phenotype, which arises fairly commonly during the course of chemotherapeutic treatment, irrespective of the tissue specificity of the primary tumor. Moreover, the expression patterns of MRP and P-glycoprotein within a given cell population have been observed to fluctuate over time. Thus, exposure to a reversal agent that interferes with P-glycoprotein function may impose selection pressure favoring the expression of MRP. Such pressure would result in continued viability of cells having a multidrug resistance phenotype. Lautier et al. (1996), 52 Biochem. Pharmacol. 967-977.

Needs remain for preventing or reversing the acquisition of a multidrug resistance phenotype in transformed cells. Particular needs remain to establish the mechanism(s) by which the multidrug resistance phenotype can be produced, and to provide additional therapies for restoring drug sensitivity to multidrug-resistant transformed cells. Still more particular needs remain to improve the clinical management of multidrug resistant tumors, especially when the multidrug resistance phenotype arises entirely or partially from overexpression of one or more genes other than those encoding P-glycoprotein or MRP.

Summary of the Invention

The present invention capitalizes on the unexpected discovery of a novel gene encoding a hitherto-unknown multidrug-resistance associated polypeptide (MRP). This novel polypeptide, designated herein as MRP-β, is encoded in the human genome and is expected to be found in the genomes of additional mammals. MRP-β likely is a transmembrane-spanning, energy-dependent transporter or pump, as are other members of the ATP Binding Cassette (ABC) Transporter Protein superfamily to which the known proteins MRP and P-glycoprotein belong. It is likely that MRP-β is disposed in the plasma membrane of a mammalian cell, and functions by ejecting intracellular substances, such as chemotherapeutic drugs. Alternatively, MRP-β may span a vesicular membrane, and function by sequestering intracellular substances. Elevated levels of expression of the novel MRP-β gene, or of bioactivity of the novel MRP-β polypeptide encoded by this gene, accordingly are expected to contribute to the emergence and/or persistence of a multidrug-resistance phenotype in transformed mammalian cells, such as carcinoma cells, including adenocarcinoma cells. Elevated expression or bioactivity of MRP-β similarly is expected to contribute to the occurrence of a multidrug-resistance phenotype in sarcoma cells and in transformed cells of the hematopoietic lineage, including leukemias, lymphomas and lymphosarcomas. MRP-β is likely to account for multidrug-resistant mammalian cell phenotypes that are refractory to treatment with reversal agents that interfere with expression, production and/or function of P-glycoprotein or of MRP.

Accordingly, it is an object of this invention to provide nucleic acids and expression vectors encoding MRP-β or a unique fragment thereof. It is another object to provide nucleic acids, including probes and antisense oligonucleotides, complementary to MRP-β encoding nucleic acids. An additional object is to provide methods and compositions for mitigating aberrant expression of an MRP-β gene, or for mitigating aberrant bioactivity of an MRP-β polypeptide. It is yet another object to provide

methods and compositions for characterizing and/or attenuating a multidrug resistance phenotype. It is still another object to provide methods and compositions, including MRP-β expressing host cells, for identifying one or more modulators, preferably inhibitors, of MRP-β. A still further object includes the modulation, preferably the inhibition, of MRP-β and of disease states associated with MRP-β. A yet further object includes the potentiation of chemotherapy to eradicate multidrug resistant transformed cells from the body of an individual, such as a cancer patient. These and other objects, along with advantages and features of the invention disclosed herein, will be apparent from the description, drawings and claims that follow.

In a first aspect, the invention features nucleic acids encoding or complementary to MRP-β or a unique fragment thereof. A preferred embodiment provides nucleic acid, the sequence of which comprises SEQ ID No: 1, an MRP-β cDNA sequence. Another preferred embodiment provided MRP-β cDNA deposited on April 16, 1997, under the terms of the Budapest Treaty, with the American Type Culture Collection (ATCC), 12301 Parklawn Drive, Rockville, MD 20852. The deposited cDNA is herein designated fohd013a05m and is accorded Deposit No. 98409. Another preferred embodiment provides ribonucleic acid (RNA) encoding an MRP-β polypeptide, the amino acid sequence of which comprises SEQ ID No: 2. Messenger RNA (mRNA) encoding MRP-β is approximately 6 kilobases (kb) in length. Other embodiments provide unique fragments (e.g., SEQ ID No: 3) of the MRP-β cDNA, including fragments corresponding to portions of the open-reading frame (ORF), and fragments corresponding to untranslated sequences 3' or 5' to the ORF. These unique fragments can be used to produce or design probes for the analysis of cellular MRP-β expression patterns, e.g., for purposes of diagnosing an abnormality in or contributed to by MRP-β. In addition, the present fragments can be used for the production or design of polymerase chain reaction (PCR) primers or antisense oligonucleotides, including therapeutic oligonucleotides that disrupt cellular MRP-β gene expression, especially abnormal or aberrant expression. It will be understood that the present nucleic acids,

especially probes and oligonucleotides, may be detectably labelled and/or may comprise one or more modifications in a nucleotide base, backbone sugar or phosphate, or be linked together by linkages other than phosphodiester bonds.

The invention is further embodied in nucleic acids that hybridize to SEQ ID No: 1 or to the complement thereof. Preferred nucleic acids hybridize to SEQ ID No: 1 or to the complement thereof under stringent conditions. Preferred antisense and/or primer oligonucleotides hybridize to unique fragments of SEQ ID No: 1 or of the complement thereof, e.g., under intracellular conditions. Additional MRP-β variant nucleic acids provided herein comprise nucleotide sequences at least 50%, preferably 60%, 70%, 80%, more preferably 90% and even more preferably 95% identical to SEQ ID No: 1. The present variant nucleic acids comprise nucleotide mutations (substitutions, deletions and/or insertions) distributed in any random or non-random frequency within the SEQ ID No: 1 sequence. The invention further provides degenerate variant nucleic acids that encode the SEQ ID No: 2 polypeptide or a unique fragment thereof. In yet further embodiments, the invention provides nucleic acids encoding variant MRP-β polypeptides, comprising amino acid sequences sharing at least 75% sequence similarity with the SEQ ID No: 2 polypeptide. Preferably, these nucleic acids encode polypeptides sharing at least 80%, 85%, 90% or more preferably 95% amino acid sequence similarity with the SEQ ID No: 2 MRP-β polypeptide. The encoded variant polypeptides comprise amino acid mutations (substitutions, deletions and/or insertions) distributed in any random or non-random frequency within the SEQ ID No: 2 sequence. "Similarity" as used herein refers to the sum of aligned amino acid residues that are identical to the corresponding SEQ ID No: 2 residues and those that are allowed point mutations therefor. Moderate gaps and/or insertions (e.g., less than about 50, preferably less than about 15, more preferably less than about 5 amino acid residues) in the aligned sequence are ignored for similarity calculation purposes. Allowed point mutations are substitutions by amino acid residues that are physically and/or functionally similar to the

corresponding aligned SEQ ID No: 2 residues, e.g., that have similar size, shape, hydrophilic or hydrophobic character, charge and chemical properties.

It should be understood that the present invention provides oligonucleotides that hybridize to any of the foregoing variant MRP-β nucleic acids, i.e., to nucleic acids that encode polypeptides comprising amino acid sequences that share at least 75% sequence similarity with the SEQ ID No: 2 polypeptide. More particularly, the invention provides olignucleotides that hybridize to one or more unique fragments of nucleic acids encoding the present MRP-β polypeptides. For therapeutic purposes and/or for PCR investigative or diagnostic purposes, the present oligonucleotides hybridize to a unique fragment comprising 5' untranslated sequence, a transcription initiation site, ORF or polypeptide coding sequence, intron-exon boundary, polyadenylation site or 3' untranslated region of the present MRP-β nucleic acids. Exemplary antisense oligonucleotides are disclosed herein (SEQ ID Nos: 4, 5, 6, 7 and 8).

For antisense-oligonucleotide based therapeutic purposes, one or more of the present antisense MRP-β oligonucleotides (optionally comprising one or more modified moieties as disclosed herein) is formulated together with a pharmaceutically acceptable vehicle to produce an antisense pharmaceutical composition suitable for local or systemic administration to a mammal, or for treatment of mammalian cells or tissue whether *in situ* or *ex vivo*. In an alternative embodiment, the present antisense oligonucleotide is encoded by an antisense expression vector comprising a nucleic acid insert complementary to the oligonucleotide sequence. The antisense vector preferably comprises or is packaged with one or more retroviral elements for infection of mammalian cells, and further comprises one or more conventional expression control elements (e.g., a promoter, transcriptional initiation site, termination site, or the like) to direct intracellular production of the antisense oligonucleotide in infected cells. The present vector also can be formulated with a pharmaceutically acceptable vehicle to produce additional antisense pharmaceutical compositions of the present invention.

Thus, the antisense vector, when internalized by a cell (e.g., by retroviral infection, pinocytosis or diffusion), directs the intracellular production of an antisense oligonucleotide which, as do any of the therapeutic antisense oligonucleotides disclosed herein, disrupts cellular expression of an MRP-β gene. Disruption of expression is achieved by interfering with MRP-β gene activation or transcription, by destabilization of MRP-β gene transcripts, or by interference with the translation of MRP-β gene transcripts. In this manner, the present invention provides compositions for mitigating aberrant expression of an MRP-β gene, e.g., expression which contributes to the emergence or persistence of a multidrug-resistant phenotype.

In a second aspect, the invention features an MRP-β polypeptide, the amino acid sequence of which comprises SEQ ID No: 2. More generally, the invention provides MRP-β polypeptides, and unique fragments (epitopes) thereof, that are encoded by any of the above-described MRP-β nucleic acids. For example, the invention provides MRP-β polypeptides, the amino acid sequences of which comprise a sequence sharing at least 75% sequence similarity (as defined herein) with SEQ ID No: 2. Such MRP-β polypeptides include naturally-occurring variants (e.g., polymorphic variants, phylogenetic counterparts of the presently disclosed human MRP-β, and/or naturally-occurring mutant variants, particularly mutants associated with the process of somatic cell transformation or tumorigenesis) and biosynthetic variants produced by routine molecular engineering techniques. Based upon an assessment of its sequence similarity to known proteins, such as MRP, the present novel MRP-β polypeptide is believed to be a novel member of the ABC Transporter Protein superfamily. Thus, it is anticipated that MRP-β polypeptides will be displayed on the surface of cells expressing an MRP-β gene, such as multidrug resistant tumor cells or transfected host cells. Of course, it is also possible that MRP-β will be incorporated into intracellular phospholipid membranes, such as vesicular membranes. Cellular production of MRP-β is expected to contribute to the emergence and/or persistence of a multidrug-resistant phenotype in transformed mammalian cells. The present invention provides various specific MRP-β

polypeptide embodiments, including MRP-β polypeptides immunogenically displayed on intact host cell membranes or cell-free membrane fractions derived from host cells; MRP-β polypeptides incorporated into synthetic or non-cellular phospholipid membranes or micelles, and MRP-β polypeptides and polypeptide fragments isolated in substantially pure form. Any of the foregoing polypeptides, or unique, immunogenic fragments (epitopes) thereof can be used to induce immune responses in human or nonhuman mammals.

Accordingly, in a third aspect, the invention features an antibody that binds selectively to an epitope unique to MRP-β. Preferably, the invention provides an antibody that binds to an MRP-β epitope that is displayed on the surface of MRP-β expressing cells, such as transformed or host cells. Antigen-binding fragments of the present antibody also are provided herein. Such fragments include truncated forms of the antibody that retain antigen binding properties, e.g., Fab, Fab_2, Fab' and Fv fragments thereof. Such fragments are produced conventionally by enzymatic or chemical cleavage of an intact antibody of the present invention. Alternatively, such fragments can be produced through molecular engineering techniques. In certain embodiments, the present antigen binding fragment is incorporated into a fusion polypeptide, such that the fragment is fused to another polypeptide, such as an immunoglobulin framework polypeptide. An exemplary framework is of human origin. Alternatively, the antigen-binding region is fused to a non-immunoglobulin polypeptide, e.g., to a cytotoxin or to a chemoattractant polypeptide. A cytotoxin polypeptide induces or mediates cell death (cytolysis), e.g., by inducing apoptosis or by disrupting cell metabolism, cell membrane integrity, intracellular fluid volume, or the like. Exemplary cytotoxic fusion proteins comprise ricin, diptheria toxin, or another naturally-sourced toxin of plant, animal or microbial origin. A chemoattractant polypeptide is any polypeptide of mammalian origin that induces or stimulates activation and localization of immune effector cells (e.g., natural killer cells, cytotoxic T cells, macrophages and the like) that typically mediate a cellular proinflammatory immune response. Exemplary

chemoattractant fusion proteins comprise a chemokine, lymphokine or cytokine polypeptide (e.g., interleukin-2 (IL2), tumor necrosis factor (TNF), and the like).

In a fourth aspect, the invention features expression vectors comprising nucleic acid encoding an MRP-β polypeptide comprising an amino acid sequence that shares at least 75% sequence similarity with SEQ ID No: 2. The nucleic acid sequence of an exemplary expression vector thus comprises SEQ ID No: 1. The nucleic acid sequence of another exemplary expression vector comprises the sequence of MRP-β cDNA deposited on even date herewith. Additional exemplary expression vectors comprise nucleic acid encoding variants, whether biosynthetic or naturally-sourced, of the presently disclosed MRP-β polypeptide. Certain embodiments of the present expression vectors encode chimeric polypeptides in which one or more MRP-β amino acid residues are substituted by the corresponding residues of another ABC Transporter Protein superfamily member, such as MRP or P-glycoprotein. Such embodiments are expected to facilitate elucidation of the molecular basis of multidrug resistance phenotypes, and thence to facilitate design or screening of novel inhibitors of multidrug resistance. In addition to nucleic acid encoding the MRP-β polypeptide, the present expression vectors comprise one or more expression control elements (e.g., promoter, transcriptional initiation site, termination site and the like) to direct the production of the encoded MRP-β polypeptide in prokaryotic or, preferably eukaryotic, host cells. Optionally, the present expression vectors further comprise a selectable marker gene. For use with eukaryotic host cells, the present expression vector may still further comprise one or more retroviral components to promote infectivity and uptake by eukaryotic, preferably mammalian, cells.

Accordingly, a fifth aspect of the present invention features a host cell transfected with an above-described expression vector. A preferred host cell displays a vector-encoded MRP-β polypeptide, comprising a sequence sharing at least 75% sequence similarity with SEQ ID No: 2, on the cell surface. A particularly preferred host

cell displays a functional and immunologically detectable MRP-β polypeptide. In other embodiments the vector encoded MRP-β polypeptide may reside within the cell, e.g., as a component of a vesicular membrane. Preferred host cells acquire a multidrug resistance phenotype and are able to eject or sequester intracellular substances, including chemotherapeutic drugs and/or metabolites thereof. The present host cells can be of human or non-human origin, and can be naturally-sourced, adapted to primary culture, or immortalized under culture conditions. Cells that are suitable for production of host cells are herein defined as source cells. Exemplary source cells include normal differentiated mammalian cells (e.g., obtained by biopsy), cells in primary culture (e.g., serially passaged benign or malignant transformed cells), and cell lines (e.g., immortalized transformed cells such as HeLa, MCF-7, and the like). Preferably, mammalian source cells are primate cells, most preferably human cells. For screening or other investigative purposes, such as the production of non-human mammals, rodent, ovine, porcine, bovine or other mammalian source cells may be used. In other embodiments, host cells can be produced from prokaryotic or eukaryotic source cells, e.g., unicellular organisms, such as yeast. Any of the foregoing can be used to produce host cells by standard cell transfection or infection techniques. Thus, an MRP-β expression vector can be stably incorporated into a source cell by transfection, pinocytosis, electroporation, microinjection, retroviral infection or the like. Transfected cells then are cultured under conditions favorable to the selective survival of MRP-β expressing host cells, e.g., in the presence of a drug cytotoxic to source cells but to which expression of MRP-β or a vector-borne selectable marker gene confers a survival advantage for host cells. Host cells so obtained are useful for the production and characterization of MRP-β antibodies, for investigation of the nature and variety of toxic substances subject to MRP-β transport, and for the screening and identification of MRP-β inhibitors as described herein.

In further embodiments, MRP-β host cells can be produced from uncommitted source cells, preferably embryonic stem cells or blastocyst cells, of non-

human mammalian origin. An uncommitted cell is one that is competent to differentiate, under appropriate conditions, into differentiated cells of one or more specific mammalian body tissues. In the present embodiment, an MRP-β expression vector is introduced into an uncommitted embryonic source cell and preferably integrates in a site-specific or nonspecific fashion into the cells' genome to produce a host cell competent to differentiate into one or a plurality of differentiated cell types. Alternatively, the present expression vector resides in the host cell as microsatellite DNA. In some embodiments, the present expression vector confers a tissue-specific pattern of MRP-β expression in tissue arising from the differentiation of uncommitted host cells. Uncommitted host cells can, through manipulation by established techniques, be used to produce non-human mammals that are either transgenic or nullizygous for MRP-β. To produce a transgenic mammal of the present invention, an above-described host embryonic stem cell or blastocyst cell is integrated (e.g., by microinjection) into a non-human mammalian blastocyst, which is thereafter implanted into the uterus of a non-human, pseudopregnant mammal, such as a mouse, rat, rabbit, sheep, goat, pig or cow. Following a normal gestation period, this intrauterine implantation procedure yields a non-human founder mammal, the body tissues of which comprise a mosaic of normal cells and host cells, the latter comprising MRP-β nucleic acid of vector origin. Progeny of the present founder mammal are characterized by germline integration of nucleic acid of vector origin. Transgenic progeny express an MRP-β polypeptide, the amino acid sequence of which comprises a sequence sharing at least 75% sequence similarity with SEQ ID No: 2. Optionally, this polypeptide is expressed in a tissue-specific manner. Thus, transgenic progeny constitutively or inducibly express MRP-β in all or a subset of their body tissues. Cells isolated or, optionally immortalized from, such transgenic tissue are expected to facilitate investigations into the discovery and characterization of MRP-β modulators useful for treatment of multidrug-resistant transformed cells arising in any mammalian body tissue. For example, transgenic progeny and/or their cells can be used to confirm whether substances initially identified

as modulators in an *in vitro* screen suppress MRP-β polypeptide production or biological function *in vitro*. Advantageously, transgenic progeny provide a tissue source that can be matched to a tissue type for which modulators of multidrug resistance are particularly desired, e.g., which has a known propensity for developing multidrug resistance. Such tissue types include, but are not limited to, mammary, respiratory tract, gastrointestinal tract, urogenital tract, hematopoietic and endocrine system tissue.

To produce a nullizygous mammal of the present invention, an above-described uncommitted source cell is transfected (e.g., infected) with a null vector, which comprises a non-expressible variant of the MRP-β encoding nucleic acid disclosed herein. The null vector further comprises sufficient nucleic acid sequence 5' and 3' to the MRP-β ORF to achieve homologous recombination with any endogenous MRP-β gene present in the source cells' genome. As a result of homologous recombination, any endogenous MRP-β gene is nullified, i.e., replaced by the present non-expressible variant. Appropriate non-expressible variants include antisense-oriented MRP-β nucleic acids, nucleic acids comprising premature stop codons in the ORF, nucleic acids comprising a defective promoter, and the like. The present null host cell is integrated into a blastocyst and implanted into a pseudopregnant mammal to produce a null founder mammal. Progeny of this founder are characterized by germline integration of nucleic acid derived from the null vector. Thus, in nullizygous progeny, the ability to express a naturally encoded MRP-β homolog is "knocked out" such that, preferably, the progeny are incapable of developing a multidrug resistance phenotype attributable to MRP-β expression. Such nullizygous progeny and/or their cells can be used to assess potential side effects or undesirable consequences of MRP-β modulator (e.g., inhibitor) therapy. Nullizygous progeny and/or their cells also can be used to detect additional genes that contribute to emergence of a multidrug-resistance phenotype, i.e., genes other than MRP-β, MRP and P-glycoprotein. Cells isolated or cultured from nullizygous progeny can be exposed to selection pressure by culturing them in the presence of a chemotherapeutic drug, and monitoring the cultures for emergence of a drug-resistant

phenotype. Optionally, the MRP-β nullizygous progeny provided herein can be cross-bred with non-human mammals nullizygous for MRP and/or P-glycoprotein. Such multiply nullizygous progeny should facilitate screening for additional genes that can contribute to the emergence of a multidrug resistance phenotype.

The above-described MRP-β compositions are useful according to teachings herein for assessing the presence of mutations in an MRP-β gene; assessing MRP-β gene expression level, especially for detecting fluctuations in expression; and, for mitigating aberrant expression and/or biological function of an MRP-β polypeptide. Preferably, the present MRP-β compositions are useful to treat a disease state or other deleterious condition contributed to by aberrant MRP-β gene expression or biological function. Most preferably, the present MRP-β compositions are useful to attenuate and/or to abrogate a multidrug resistant phenotype, e.g., of transformed cells in the body of a cancer sufferer. As a result, the present invention offers means for potentiating chemotherapy to eradicate multidrug-resistant transformed cells from an individual's body.

Thus, in a sixth aspect, the invention features diagnostic methods for detecting abnormalities in an MRP-β gene. In one embodiment, the invention provides a method of detecting a mutation or other structural abnormality in an MRP-β gene. Mutations, whether of germline or somatic origin, may indicate whether the process of cell transformation (tumorigenesis) has been initiated or is likely to arise in an individual's tissues. Mutations are detected by obtaining cellular tissue from a mammal, preferably a human, suspected of harboring a variant MRP-β gene, and treating the tissue so as to release nucleic acids therefrom. Preferably the cellular tissue is obtained from a body tissue suspected of comprising transformed cells. Thus, the present method provides information relevant to diagnosis of the presence of a tumor. The method may be practiced with any body tissue type which comprises cells, including body fluid cell suspensions (e.g., blood, lymph, cerebrospinal fluid, peritoneal fluid or ascites fluid).

Released cellular nucleic acids are combined, under hybridization conditions, with an oligoucleotide of the present invention, e.g., an oligonucleotide complementary to nucleic acid encoding MRP-β. Preferably, the oligonucleotide is complementary to a unique fragment of the full-length MRP-β nucleic acid. Following incubation with the oligonucleotide under suitable hybridization conditions, the released nucleic acids are assayed for formation of a hybrid comprising the oligonucleotide. In a preferred embodiment wherein the oligonucleotide is complementary to SEQ ID No: 1 or a unique fragment thereof, formation of the hybrid confirms that the individual harbors at least one wild-type MRP-β gene allele (comprising SEQ ID No: 1). Failure to form a hybrid under stringency conditions that do not tolerate base pair mismatching confirms that the individual lacks a wild-type allele, i.e., that the individual harbors an aberrant, e.g., mutant, variant of the MRP-β gene.

In another embodiment, the invention provides a method of assessing expression, especially aberrant expression, of a cellular MRP-β gene. As with the preceding embodiment, aberrant expression may indicate the presence, persistence or reappearance of multidrug-resistant tumor cells in an individual's tissue. More generally, aberrant expression may indicate the occurrence of a deleterious or disease-associated phenotype contributed to by MRP-β. MRP-β gene expression is assessed by obtaining a sample of cellular tissue from a mammal (e.g., a human), preferably from a body site implicated in a possible diagnosis of diseased or malignant tissue, and treating the tissue to release RNA therefrom. Cellular RNA is combined with an MRP-β oligonucleotide generally as described above, and the resulting mixture is assayed for the presence of a hybrid comprising the MRP-β oligonucleotide and a cellular MRP-β gene transcript. In preferred embodiments, the presence and/or relative abundance of this hybrid is expected to indicate aberrant expression of a cellular MRP-β gene, and to correlate with the occurrence *in situ* of transformed cells, especially transformed cells having a multidrug-resistant phenotype.

Preferably, the foregoing embodiments can be practiced using a detectably labeled or otherwise modified MRP-β oligonucleotide, most preferably with an oligonucleotide comprising a peptide-nucleic acid backbone.

In yet another embodiment, the invention provides a diagnostic method using an above-described antibody or fragment thereof to characterize aberrant MRP-β associated phenotype, e.g., drug-resistant phenotype of a transformed cell. This method involves obtaining cellular tissue from a mammal (e.g., a human) suspected of harboring transformed cells, and contacting the tissue with an above-described antibody under conditions such that, if cells of the obtained tissue display a recognized epitope unique to MRP-β, an antibody-epitope complex forms. Generally, the method is practiced with intact cells. The practitioner may, however, desire to generate a more sensitive assay for total cellular MRP-β content. In these circumstances, the method is practiced with permeabilized or solubilized cells, which can be produced by exposing the cells to heat, mechanical disruption, detergent, hypo- or hyper-osmotic conditions, and like conventional techniques. After a sufficient period of time has elapsed for formation of the antibody-epitope complex, the tissue is assayed for presence of the complex, formation or abnormal elevation of which indicates presence in the tissue of cells abnormally expressing MRP-β. As disclosed herein, such cells are likely transformed cells characterized by a drug-resistance phenotype.

Information obtained from practice of the foregoing diagnostic methods is expected to be useful in prognostication, staging and clinical management of diseases and other deleterious conditions affecting an individual's health status. In preferred embodiments, the foregoing diagnostic methods provide information useful in prognostication, staging and management of malignancies (tumors) that are characterized by expression of MRP-β and thus by a multidrug-resistance phenotype. The information more specifically assists the clinician in designing chemotherapeutic or other treatment regimes to eradicate such malignancies from the body of an afflicted

mammal, typically a human. The present methods can be practiced with any samples of any body tissue type, and are desirable for assessing cellular tissue of mammary, respiratory tract, urogenital tract, endocrine system or immune system origin. The present methods are particularly useful to assess breast biopsy, bronchoalveolar lavage, ovarian, uterine or cervical biopsy, prostate or testicular biopsy, pancreatic biopsy, and spleen, bone marrow or lymph node biopsy samples.

Further general aspects of the invention feature therapeutic methods and compositions, including one or more modulators (stimulators or, preferably, inhibitors) of the expressed MRP-β gene and/or protein. Accordingly, the invention provides means for mitigating (detectably decreasing or otherwise affecting) aberrant expression of an MRP-β gene, or aberrant production or biological function of an MRP-β polypeptide. The invention thus provides means for attenuating an undesirable phenotype, such as a disease-associated phenotype, that is contributed to by MRP-β. In preferred embodiments, the invention provides means for attenuating a multidrug-resistance phenotype, particularly a phenotype contributed to by MRP-β. More particularly, a seventh aspect of the invention features methods for mitigating aberrant expression of an MRP-β gene, and/or aberrant alteration or biological function of an MRP-β polypeptide. One embodiment involves the administration of an antisense pharmaceutical composition of the present invention to a mammal suffering from effects of the aberrant phenotype associated with altered expression and/or function of MRP-β. Another embodiment involves the administration of an antibody or fusion polypeptide of the present invention. In either embodiment, the therapeutic agent is administered systemically or locally under conditions sufficient to mitigate or attenuate the aberrant MRP-β associated phenotype. Preferably, the therapeutic agent is administered under conditions sufficient to destroy cells aberrantly producing MRP-β. In this manner, the invention provides means for destroying multidrug-resistant tumor cells *in situ* in the body of a mammal. In preferred embodiments, either of the foregoing therapeutic agents can be administered as an adjuvant to conventional chemotherapy. That is, either of the

foregoing therapeutic agents can be coadministered together with one or more chemotherapeutic drugs. The present antisense or fusion polypeptide therapeutic agent can be administered prior to, concomitant with, or following administration of one or more chemotherapeutic drugs. In such embodiments, the antisense pharmaceutical composition mitigates resistance of MRP-β expressing cells to the cytotoxic effects of the chemotherapeutic drug. That is, the antisense composition attenuates the MRP-β phenotype, which is expected to be characterized by display of an ABC Transporter Protein family member (MRP-β) and by the property of multidrug resistance. This is accomplished by disrupting activation or transcription of the MRP-β gene, or by destabilizing RNA transcripts thereof. Diminished or discontinued expression of MRP-β renders cells more susceptible to the cytotoxic effects of a chemotherapeutic drug that otherwise would be exported by MRP-β. Similarly, a therapeutically administered cytotoxic fusion polypeptide localizes in the vicinity of cells aberrantly displaying MRP-β, producing cytolysis thereof. A chemoattractant fusion polypeptide also localizes to MRP-β displaying cells, stimulating destruction thereof by macrophages, killer T cells or cytotoxic T cells.

An eighth aspect of the invention features methods for identifying a modulator (a stimulator or, preferably, an inhibitor) of MRP-β. The present modulator is useful for treating a disease or deleterious condition that is contributed to by MRP-β. Preferably, the modulator is a small molecule. In general, the present identification method relies on the use of an MRP-β expressing host cell produced as described herein. Prokaryotic or eukaryotic host cells can be used for purposes of identifying an MRP-β modulator; however in general, eukaryotic host cells are preferred. Yeast or mammalian cells may be used, as desired or as dictated by specific circumstances. Presently, mammalian host cells, particularly human cells are preferred. The MRP-β expressing host cell is contacted with a candidate modulator, and after a sufficient period of time for modulatory effects to be manifested, the cell is assayed to determine whether the candidate indeed affects MRP-β. In one embodiment, the level of cellular MRP-β gene

expression is assayed. A detectable decrease (attenuation) or cessation (abrogation) in MRP-β gene expression indicates that the candidate is an inhibitory modulator or inhibitor. Conversely, a detectable increase (augmentation) in MRP-β gene expression indicates that the candidate is a stimulatory modulator or stimulator. Another embodiment involves assay of the amount or rate of production of MRP-β polypeptide displayed by the cell. A detectable decrease or cessation of immunologically recognized MRP-β polypeptide indicates that the candidate is an inhibitory modulator. In a third embodiment, the host cell is contacted with a substrate (e.g., a cytotoxin) exported or sequestered by MRP-β. The candidate inhibitor is contacted with the host cell prior to, concomitantly with, or following exposure to the substrate. The amount of substrate exported or sequestered by the cell is assessed. A detectable decrease in efflux or sequestration of the substrate indicates that the candidate is an inhibitory modulator. Alternatively, in specific embodiments wherein the substrate is cytotoxic, survival of the host cell is assessed. A detectable decrease in survival indicates that the candidate is an inhibitory modulator. Candidate substances appropriate for screening as MRP-β modulators in any of the foregoing embodiments include natural or synthetic metabolites, toxins, antibiotics, elements of a combinatorial chemistry, nucleotide or peptide library, naturally sourced cell secretion products, cell lysates, and the like. Preferred substances for screening, and preferred modulators, are small molecules.

Accordingly, a ninth aspect of the invention features an MRP-β modulator, especially an inhibitory modulator, identified by any of the above-described methods. Preferably, the modulator is a small molecule, e.g., an element of a combinatorial chemistry library or a low molecular weight natural or synthetic product or metabolite. The modulator may be dispersed in a pharmaceutically acceptable vehicle to produce a multidrug-resistance attenuating pharmaceutical composition of the present invention.

A tenth aspect of the invention thus features modulator-based methods of mitigating aberrant MRP-β expression and/or polypeptide production and/or biological

function. The present method involves the step of administering an MRP-β modulator, optionally dispersed in a pharmaceutically acceptable vehicle to a mammal suffering from effects of the MRP-β associated aberrancy. Therapeutic modulation (preferably inhibition) of MRP-β is useful for the treatment, including prophylaxis, remediation and palliation, of any disease or deleterious condition that is contributed to by an abnormality affecting the MRP-β gene, its expression, MRP-β polypeptide production or biological function. In a preferred embodiment, the invention provides a method for improving (potentiating) effectiveness of chemotherapy to eradicate aberrant MRP-β expressing cells, e.g., multidrug resistant transformed cells, from the body of a mammal. This method involves the steps of administering a chemotherapeutic drug to the mammal, and coadministering an MRP-β modulator identified as described herein. Preferably, the modulator is provided in the form of a multidrug-resistance attenuating composition, i.e., dispersed in a pharmaceutically acceptable vehicle. This method is particularly preferred where a chemotherapy adjuvant is desired to eradicate multidrug-resistant tumor cells. Advantageously, the method can be practiced where a fluid (e.g., leukemia, lymphoma, lymphsarcoma or ascites) tumor is present, or where the situs of a primary or metastatic tumor is deemed unsuitable for surgical intervention or especially where a remontant or reemergent tumor is observed following an initial course of chemotherapeutic treatment. The present embodiments are suitable for the treatment of any tumor, especially of mammary, respiratory tract, urogenital tract, endocrine system or immune system origin.

Brief Description of the Drawings

The foregoing and other objects, features and advantages of the present invention, as well as the invention itself, will be more fully understood from the following description of preferred embodiments, when read together with the accompanying drawings, in which:

FIGURE 1 is a text representation of an MRP-β cDNA sequence and of the polypeptide sequence encoded therein, as set forth in SEQ ID Nos: 1 and 2.

FIGURE 2 is a text representation comprising aligned amino acid sequences of the known ABC Transporter Protein superfamily member MRP (described in Deeley et al. (1996) U.S. Patent 5,489,519), and of the novel MRP-β disclosed herein. Dashes (-) indicate gaps introduced to maximize alignment of similar sequences; colons (:) indicate the locations of identical aligned amino acid residues.

Detailed Description of Preferred Embodiments

Mammalian cells having a "multidrug-resistance" or "multidrug-resistant" phenotype are characterized by the ability to sequester, export or expel a plurality of cytotoxic substances (e.g., chemotherapeutic drugs) from the intracellular milieu. Cells may acquire this phenotype as a result of selection pressure imposed by exposure to a single chemotherapeutic drug (the selection toxin). Alternatively, cells may exhibit the phenotype prior to toxin exposure, since the export of cytotoxic substances may involve a mechanism in common with normal export of cellular secretion products, metabolites, and the like. Multidrug resistance differs from simple acquired resistance to the selection toxin in that the cell acquires competence to export additional cytotoxins (other chemotherapeutic drugs) to which the cell was not previously exposed. For example, Mirski et al. (1987), 47 Cancer Res. 2594-2598, describe the isolation of a multidrug-resistant cell population by culturing the H69 cell line, derived from a human small cell lung carcinoma, in the presence of adriamycin (doxorubicin) as a selection toxin. Surviving cells were found to resist the cytotoxic effects of anthracycline analogs (e.g., daunomycin, epirubicin, menogaril and mitoxantrone), acivicin, etoposide, gramicidin D, colchicine and *Vinca*-derived alkaloids (vincristine and vinblastine) as well as of adriamycin. Similar selection culturing techniques can be applied to generate additional multidrug-resistant cell populations.

The functional property of multidrug-resistance is associated with expression and cell-surface display of one or more ABC Transporter Protein superfamily members with energy-dependent export function (e.g., P-glycoprotein, MRP or MRP-β as disclosed herein). The cell population described in Mirski et al. (1987) was reported in Cole et al. (1992), 258 Science 1650-1654 to overexpress MRP (a correction of the reported MRP sequence appears at 260 Science 879). Currently, antibodies specifically reactive with P-glycoprotein or MRP, or nucleic acid probes specific for the corresponding expressed nucleic acid sequences, are used to ascertain the molecular basis of multidrug-resistance in a given cell population. Where the cell population in question includes transformed cells in the body of a cancer sufferer, determination of the molecular basis of the observed phenotype can assist the clinician in ascertaining whether treatment with one of the so-called "chemosensitizers" or "MDR reversal agents," the majority of which affect P-glycoprotein, is appropriate. Thus, knowledge of the molecular basis of the observed phenotype provides information relevant to developing or revising a course of disease management. Zaman et al. (1993), 53 Cancer Res. 1747-1750, cautions, however, that the induction or overexpression of MRP does not account for all forms of multidrug-resistance phenotype that are not attributable to P-glycoprotein expression. The discovery of MRP-β, reported herein, establishes that additional members of the ABC Transporter Protein family exist in the mammalian (e.g., human) genome and likely contribute to the occurrence of multidrug-resistance in transformed cells.

MRP-β was identified by computer-assisted screening of a nucleic acid sequence database corresponding to a human endothelial cell cDNA library. The library comprises cDNAs derived from RNA transcripts of genes expressed by differentiated endothelial cells cultured from microvascular tissue of mammary origin. The library was constructed, and nucleic acid components thereof were sequenced, by conventional techniques as set forth in Current Protocols in Molecular Cloning, Ausubel et al., eds. (1989), Greene Publishing and Wiley Interscience, New York, NY. The known

sequence of MRP was used to query the database using the TBLAST N algorithm disclosed in Altschul et al. (1990), 215 J. Mol. Biol. 403-410. The query sequence is disclosed in Cole et al. (1992), 258 Science 1650-1654 and 260 Science 879. See also Seq ID No: 1 of Deeley et al. (1996), U.S. Patent 5,489,519, the disclosure of which is incorporated by reference herein. The starting search parameters for TBLAST N were as follows: score = 200; word length = 12.

The foregoing analysis identified a novel nucleic acid sequence with detectable similarity to the query sequence. The novel sequence, disclosed herein as SEQ. ID No: 3, corresponds to a unique fragment of a hitherto unknown multidrug-resistance associated polypeptide, herein designated MRP-β. As defined herein, a "unique fragment" of a protein or nucleic acid is a peptide or oligonucleotide of sufficient length to have a sequence unique to a particular gene or polypeptide, i.e., a sequence not shared by related or unrelated genes or polypeptides. Thus, for example, a unique nucleic acid fragment typically will have at least 16 nucleotide residues, and a unique polypeptide fragment typically will have at least 6 amino acid residues. Preferably, to ensure substantially unique occurrence in a typical higher eukaryotic genome, a unique nucleic acid fragment should have at least 20 nucleotide residues, and a unique polypeptide fragment should have at least 8 amino acid residues. Unique polypeptide fragments are referred to herein as epitopes. The SEQ ID No: 3 unique fragment of MRP-β nucleic acid is 465 nucleotide residues in length and has a sequence approximately 62% identical to that of the corresponding aligned fragment of the MRP gene. In contrast, SEQ ID No: 3 lacks detectable similarity to the product of the MDR1 gene, P-glycoprotein.

A nucleic acid probe was prepared using the SEQ ID No: 3 sequence, as described in EXAMPLE 1 herein, and used for hybridization screening of an appropriate expression (cDNA) library. The screen yielded an MRP-β cDNA having the sequence set forth as nucleotides 67-4847 of SEQ ID No: 1 herein. This cloned cDNA has been designated fohd013a05m and has been deposited (April 16, 1997) in the American Type

Culture Collection under the terms of the Budapest Treaty. The sequence of fohd013a05m accordingly is incorporated herein by reference. The original SEQ ID No: 3 fragment corresponds generally to nucleotides 3701 to 4144 of the cloned SEQ ID No: 1 cDNA. The full-length MRP-β cDNA extends a short distance upstream (5') of the fohd013a05m MRP-β insert. The MRP-β transcript produced in human cells and/or tissue is approximately 6 kb, as visualized in the Northern blot studies described in EXAMPLES 2 and 3. A cDNA comprising 66 nucleotides upstream (5') of the fohd013a05m MRP-β insert was isolated as described in EXAMPLE 1. The cDNA sequence presented in SEQ ID No: 1 comprises the sequence of the fohd013a05m MRP-β insert and the 66 upstream nucleotides. The native 5' end of the cellular MRP-β transcript can also be elucidated readily using a 5'-RACE protocol known in the art, for example as described in Siebert et al. (1995), 23 Nucl. Acids Res. 1087-1088, and in the Clonetech, Inc. User Manual for Marathon-Ready cDNA (1996), the teachings of which are incorporated herein by reference.

The present invention encompasses all MRP-β nucleic acids that can be isolated or constructed by conventional molecular engineering techniques, using the information made available as a result of the present disclosure. Thus, for example, the invention encompasses nucleic acids comprising sequences complementary to all or a unique fragment of the SEQ ID No: 1 cDNA. The sequence of a "complementary" nucleic acid strand is composed of the Watson-Crick base pair partners of the nucleotide residues in a specified nucleic acid, i.e., a guanidine (G) residue corresponding to each cytosine (C) residue in the specified nucleic acid, and an adenine (A) residue corresponding to each thymidine (T) or uracil (U) residue therein. Thus, the invention encompasses RNA having a sequence complementary to SEQ ID No: 1. The present RNA can be obtained as a cell-free lysate or extract (e.g., as described in EXAMPLE 2), or can be isolated in substantially pure form using techniques described in Current Protocols in Molecular Cloning, Ausubel et al., eds. (1989), Greene Publishing and Wiley Interscience, New York, NY and in Sambrook et al. (1989), Molecular Cloning: A

Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. The invention further encompasses a nucleic acid probe or primer having a nucleotide sequence complementary to a unique fragment of the MRP-β gene described herein. The probe optionally further comprises a detectable moiety, or creates a detectable complex, when hybridized to the target (MRP-β) sequence. Non-limiting examples of appropriate detectable moieties including fluorophores (e.g., fluorescein, rhodamine, Texas Red and the like), radionucleotides (e.g., ^{3}H, ^{14}C, ^{32}P and the like), and binding-pair partners (e.g., biotin, avidin or streptavidin). The probe or primer need not be strictly complementary to the target sequence: it is only necessary that a sufficient number of probe nucleotides be capable of forming base pairs with target nucleotides to produce a stable, double-stranded nucleic acid complex under hybridization conditions.

Hybridization is the noncovalent, antiparallel bonding of complementary nucleic acid strands, in which Watson-Crick base pairing is established. To ensure specificity, hybridization should be carried out under stringent conditions, defined herein as conditions of time, temperature, probe length, probe and/or target concentration, osmotic strength, pH, detergent, carrier nucleic acid, etc. that permit no more than an occasional base-pairing mismatch within a probe/target duplex. Highly stringent conditions exclude all but about one base pair mismatch per kb of target sequence. Exemplary highly stringent conditions involve hybridization to membrane immobilized target nucleic acid at a temperature of 65°C in the presence of 0.5 m $NaHPO_4$, 7% SDS, 1mM EDTA, followed by washing at 68°C in the presence of 0.1x SSC, 0.1% SDS. Current Protocols in Molecular Biology (1989), Ausubel et al., eds., Greene Publishing and Wiley Interscience, New York, NY. In circumstances where relatively infrequent mismatches, e.g., up to about ten mismatches per kb of target, can be tolerated, moderately stringent conditions may be used. For moderate stringency, probe/target hybrids formed under the above conditions are washed at 42°C in the presence of 0.2x

SSC, 0.1% SDS. The invention encompasses all nucleic acids that hybridize to nucleic acid, the sequence of which comprises SEQ ID No: 1 or a unique fragment thereof.

Nucleic acids that are complementary to or hybridize to all or a unique fragment of the novel MRP-β gene can be used as antisense or primer oligonucleotides. Antisense oligonucleotides disrupt gene expression and/or protein production and thereby attenuate an aberrant phenotype attributable to inappropriate expression or activation of the target gene. Lautier et al. (1996), 52 Biochem. Pharmacol. 967-977. As a result, the phenotype is abrogated or its penetrance is diminished (attenuated). Therapeutic intervention to attenuate a multidrug-resistance phenotype, for example, restores cellular vulnerability to cytotoxic drugs. Smyth et al. (1996), PCT Publ. WO 96/02556, teaches that antisense oligonucleotides disrupt expression of the target gene by interfering with gene transcription, transcript splicing, or translation; by triggering enzymatic destruction by RNAse H; or by destroying the target through one or more reactive moieties incorporated into the antisense compound. Preferred oligonucleotides herein have sequences sufficiently complementary to all or a unique fragment of the MRP-β gene to hybridize, under intracellular conditions, to the gene's coding or noncoding strand, or to an RNA transcript of the gene. Optionally, the oligonucleotide can be designed to hybridize to a polypeptide coding region, or to a 5' or 3' untranslated region of the gene or gene transcript, or to a gene intron or an intron/exon boundary. Typically, the present oligonucleotides are at least 9 nucleotides in length, and range from about 12 to about 40 bases in length, and are generally about 16 to 30 bases in length, with about 20 bases being considered optimal. Exemplary oligonucleotides are at least 15, 21, or 24 nucleotides in length. Specific examples of the present oligonucleotides are set forth in SEQ ID Nos: 4, 5, 6, 7 and 8. These and other exemplary oligonucleotides can be synthesized readily by conventional techniques.

While either DNA or RNA is suitable for use in primer, probe or antisense oligonucleotides, it is often desirable to include one or more modified bases, backbone sugar moieties, or backbone linking groups. Thus, Smyth et al. (1996) teaches that

alkylphosphonates, phosphorothioates, phosphorodithioates, phosphate esters, alkylphosphonothioates, phosphoramidates, carbamates, carbonates, phosphate triesters, acetamidate, 2-O-methyls, and carboxymethyl esters all are suitable for use in the context of antisense oligonucleotides. Preferred modified olignoculeotides herein comprise a modified backbone structure. Peptide nucleic acid (PNA) oligonucleotides prepared according to the teachings of Perry-O'Keefe et al. (1996), 93 Proc. Nat'l. Acad. Sci. USA 14670-14675, and Egholm et al. (1993), 365 Nature 566-568, are particularly preferred herein.

In addition, the invention encompasses all MRP-β nucleic acids having sequences at least 50% identical to SEQ ID No: 1 or to the complement thereof. The determination of whether a particular sequence meets this criterion is made using the TBLAST N algorithm according to the teachings of Altschul et al. (1990), 215 J. Mol. Biol. 403-410, the teachings of which are incorporated herein by reference. Such nucleic acids encode variants, which may be naturally-occurring or biosynthetic, of the MRP-β polypeptide disclosed herein.

To determine the percent homology of two amino acid sequences or of two nucleic acids, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are homologous at that position (i.e., as used herein amino acid or nucleic acid "homology" is equivalent to amino acid or nucleic acid "identity"). The percent homology between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100). The determination of percent homology between two sequences can be accomplished using a mathematical algorithim. A preferred, non-limiting example of a

mathematical algorithim utilized for the comparison of two sequences is the algorithm of Karlin and Altschul (1990) Proc. Natl. Acad. Sci. USA 87:2264-68, modified as in Karlin and Altschul (1993) Proc. Natl. Acad. Sci. USA 90:5873-77. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul, et al. (1990) J. Mol. Biol. 215:403-10. BLAST nucleotide searches can be performed with the NBLAST program, score = 100, wordlength = 12 to obtain nucleotide sequences homologous to D6 nucleic acid molecules of the invention. BLAST protein searches can be performed with the XBLAST program, score = 50, wordlength = 3 to obtain amino acid sequences homologous to D6 protein molecules of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., (1997) Nucleic Acids Research 25(17):3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., XBLAST and NBLAST) can be used. See http://www.ncbi.nlm.nih.gov. Another preferred, non-limiting example of a mathematical algorithim utilized for the comparison of sequences is the algorithm of Myers and Miller, CABIOS (1989). Such an algorithm is incorporated into the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used.

Similarly, the invention encompasses all nucleic acids which, by virtue of the well-known degeneracy of the genetic code, also encode the SEQ ID No: 2 polypeptide. Such degenerate variants may be naturally-occurring or may be produced through routine application of molecular engineering techniques. Current Protocols in Molecular Cloning, Ausubel et al., eds. (1989), Greene Publishing and Wiley Interscience, New York, NY and in Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Furthermore, the invention encompasses all nucleic acids encoding polypeptides having sequences that share at least 75% sequence similarity with the disclosed MRP-β polypeptide.

Similarity is calculated generally according to the method of Altschul et al. (1990), 215 J. Mol. Biol. 403-410, using the TBLAST P algorithm. Moderate gaps or insertions of amino acid residues are ignored for similarity calculation purposes. Preferably, the MRP-β variants encoded by these nucleic acids function similarly to MRP-β when expressed by a host cell produced as described herein. That is, preferred MRP-β variant polypeptides are displayed on the surface of a host cell and contribute to the cell's acquisition of a multidrug-resistance phenotype. MRP-β variants thus may differ from that comprising SEQ ID No: 2 by the presence of one or more amino acid insertions, deletions, or point substitutions. Deletion variants are expected to facilitate investigation into the minimum MRP-β polypeptide structure required to support drug transport and thus multidrug-resistance phenotype. Substitution variants are expected to facilitate investigation into the mechanism and specificity of MRP-β function. Exemplary substitution variants include chimeric polypeptides in which one or more MRP-β amino acid residues are replaced by the corresponding residue in either the MRP or P-glycoprotein sequence. All nucleic acids encoding such variants are within the scope of the present invention. All oligonucleotides complementary to, or which hybridize to, the present nucleic acids are within the scope of this invention.

All of the foregoing nucleic acids of the present invention can be produced, expressed, and/or manipulated by conventional molecular engineering techniques such as the techniques set forth in Current Protocols in Molecular Cloning, Ausubel et al., eds. (1989), Greene Publishing and Wiley Interscience, New York, NY and in Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, and the teachings described and referenced in Watson et al. (1992), Recombinant DNA, 2nd ed., Scientific American Books and W.H. Freeman & Co., New York, NY.

Any of the foregoing nucleic acids can be inserted into an expression vector by routine molecular engineering techniques. Current Protocols in Molecular Cloning, Ausubel et al., eds. (1989), Greene Publishing and Wiley Interscience, New York, NY

and in Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, and publications referenced in Watson et al. (1992), Recombinant DNA, 2nd ed., Scientific American Books and W.H. Freeman & Co., New York, NY. Preferred expression vectors thus encode full-length or unique fragment MRP-β polypeptides. Particularly preferred are expression vectors that, when expressed in a suitable host cell, contribute to the emergence of a multidrug-resistance phenotype therein. In other embodiments, the vector comprises DNA or RNA complementary to an antisense oligonucleotide. The present expression vectors further comprise one or more conventional expression control elements, such as an enhancer, promoter, initiation site, or termination site operatively associated with the inserted MRP-β nucleic acid. Non-limiting examples of suitable expression control elements include the cytomegalovirus immediate early gene, the early or late promoters of SV40 adenovirus, the lac system, the trp system, the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoter of acid phosphatase, the promoters of yeast α-mating factors, and immunoglobulin enhancers and/or promoters. Optionally, the expression vector may comprise a selection marker, such as an antibiotic resistance gene. Single or multiple copies of the inserted MRP-β nucleic acid can be encoded by the vector. Preferably, for production of eukaryotic (preferably mammalian) host cells, or for therapeutic purposes, the vector is retroviral in origin or comprises one or more retroviral elements. The vector can be taken up (internalized) by cells *via* transfection, infection, microinjection, pinocytosis or in the course of cell division, or can be packaged, e.g., in a liposome or retroviral envelope. In this manner, the vector can be designed for selective internalization in dividing cells, transformed cells, or in cells of a tissue type susceptible to retroviral infection. Deeley et al. (1996), U.S. Patent 5,489,519, the teachings of which are incorporated herein by reference, summarizes conventional techniques for the preparation of expression vectors.

The present MRP-β expression vectors are suitable for use in any conventional host cell transfection technique, e.g., as described in Current Protocols in Molecular Cloning, Ausubel et al., eds. (1989), Greene Publishing and Wiley Interscience, New York, NY and in Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, and in publications referenced in Watson et al. (1992), Recombinant DNA, 2nd ed., Scientific American Books and W.H. Freeman & Co., New York, NY. Thus, the present invention further provides a host cell that produces an MRP-β polypeptide or an MRP-β antisense oligonucleotide. Preferred host cells display an MRP-β polypeptide on the cell surface and/or display a multidrug-resistance phenotype. Such host cells are expected to facilitate elucidation of the types or structural classes of chemotherapeutic drugs or other substances ejected or sequestered from the intracellular milieu by MRP-β. Thus, MRP-β host cells allow rapid, *in vitro* evaluation of the specific characteristics of the multidrug-resistance phenotype associated with MRP-β expression or overexpression. Such cells further allow production of MRP-β polypeptides and antibodies as described below.

Cells (source cells) suitable for the production of the foregoing host cells include, but are not limited to, primary or immortalized epithelial cells such as carcinoma cells or cell lines. Additional source cells include primary or immortalized mesenchymal cells, such as sarcoma cells. Still further suitable cells include hematopoietic system cells, such as leukemia, lymphoma or lymphosarcoma cells. Mammalian or non-mammalian cells can be used, but in general, mammalian (e.g., murine, ovine, porcine, bovine or, preferably, human) cells are preferred. For certain purposes, such as the rapid phenotypic characterization of deleterious phenotypes (e.g., multidrug resistance phenotypes) conferred by MRP-β alteration, expression or overexpression, or such as the rapid screening of candidate modulators of MRP-β, non-mammalian cells such as insect cells or yeast cells, also may be used. In all circumstances, the identification of transfectants (newly produced host cells) is

dependent on the use of source cells that are vulnerable to the cytotoxic effects of drugs transported by MRP-β or metabolized by the product of a selection marker gene optionally included in the vector.

Deeley et al. (1996), 5,489,519, Cole et al. (1994), 54 Cancer Res. 5902-5910, and Stride et al. (1995), 49 Mol. Pharmacol. 962-971, each describe the transfection of human HeLa cells with MRP to produce an MRP expressing host cell. Engel et al. (1996), U.S. Patent 5,556,856, and Zelle et al. (1996), U.S. Patent 5,543,423, describe the transfection of murine leukemia cells with MDR-1 to produce P-glycoprotein expressing host cells. Sarkadi et al. (1995), PCT Publ. WO9531474 describes the transfection of murine NIH 3T3 fibroblasts and of *Spodoptera frugiperda* (insect) cells with MDR-1 to produce P-glycoprotein expressing host murine and insect cells, respectively. Ruetz et al. (1996), 271 J. Biol. Chem. 4154-4160, describes the transfection of *Saccharomyces cerevisiae* (yeast) with MRP and MDR-1 to produce yeast host cells. Any of the above-mentioned, available source cells can be transfected according to standard techniques with an MRP-β expression vector to produce MRP-β expressing host cells. Relevant techniques are disclosed in the above-cited references and in Current Protocols in Molecular Cloning, Ausubel et al., eds. (1989), Greene Publishing and Wiley Interscience, New York, NY. Currently, the immortalized MCF-7 human breast adenocarcinoma cell line, available from the American Type Culture Collection as ATCC No. HTB22, is a preferred source cell. An exemplary standard transfection technique suitable for use with MCF-7 is the lipofectin technique summarized in Cole et al. (1994), however, many conventional alternatives (e.g., calcium phosphate; lithium acetate; baculoviral or retroviral infection) are available and can be used with the MCF-7 or other exemplary source cell lines. After transfection, transfectants can be identified by culturing the cells in the presence of hygromycin B (as in Cole et al. (1994)) or another selection toxin, such as bisantrene or adriamycin (doxorubicin). Expression of a biologically-functional MRP-β polypeptide can be confirmed by analyzing cellular RNA for the presence of vector-derived MRP-β

transcripts; by analyzing cellular protein for the presence of an epitope unique to MRP-β; by analyzing the cell surface for display of an epitope unique to MRP-β; or, by analyzing whether the cell has acquired an MRP-β associated phenotype, such as a multidrug-resistance phenotype.

The present host cells initially are expected to facilitate production of MRP-β polypeptides and structural and functional analysis thereof. The MRP-β polypeptide comprising SEQ ID No: 2 is expected to bind ATP, and to be an integral, multispanning transmembrane protein generally as described in Almquist et al. (1995), 55 Cancer Res. 102-110. A significant portion of the total MRP-β produced in host cells is expected to span the cells' plasma membrane, with an additional portion being present intracellularly, e.g., in the endoplasmic reticulum and/or the Golgi apparatus. Thus, MRP-β host cells are expected to display extracellular portions of the multispanning MRP-β polypeptide on the cell surface, appropriately configured to mediate the ATP-dependent sequestration or export (efflux) of a plurality of cytotoxic drugs, including drugs conventionally used as chemotherapeutic agents. These general properties are deduced from an assessment of the primary structure (sequence) of the MRP-β polypeptide. MRP-β is considered to be a novel member of the ABC Transporter Protein superfamily and is deemed likely to contribute to multidrug-resistance phenotypes by mediating drug transport across cellular phospholipid membranes. FIGURE 2 sets forth an exemplary sequence alignment of the disclosed novel MRP-β polypeptide (SEQ ID No: 2), with relevant sequence of the MRP polypeptide of Deeley et al. (1996), U.S. Patent No. 5,489,519.

The present host cells provide an appropriate purification source for obtaining useful quantities of MRP-β polypeptide. The polypeptide can be isolated in substantially pure form (i.e., essentially free of detectable levels of non-MRP-β polypeptides or other cell components) by an appropriate combination of one or more protein extraction or purification techniques such as those described in Sambrook et al.

(1989), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Alternatively, an MRP-β enriched subcellular membrane preparation can be obtained by suitable cell disruption and fractionation techniques, all of which are well-known in the art. An exemplary, adaptable protocol for obtaining an MRP enriched subcellular membrane preparation is set forth in Zaman et al. (1994), 91 Proc. Natl. Acad. Sci. USA 8822-8826. Intact host cells, MRP-β enriched membrane preparations thereof, and/or isolated MRP-β protein can be used for a number of purposes, such as the production of monoclonal or polyclonal antibodies, characterization of substrates affected by MRP-β biological function, and identification of novel modulators (e.g., inhibitors) affecting MRP-β biological function.

Antibody production involves administration of one or more immunogenic doses of an MRP-β polypeptide preparation (whether isolated or incorporated in a cell membrane) to an appropriate non-human animal, such as a mouse, rat, rabbit, guinea pig, turkey, goat, sheep, pig, or horse. To enhance immunogenicity, the preparation can be emulsified with a conventional adjuvant, such as Freund's complete or incomplete adjuvant. Routine monitoring of serum immunoglobulins, using peripheral blood samples withdrawn at appropriate intervals (e.g., seven to ten days) after an initial or subsequent immunization, can be used to detect the onset and/or maturation of a humoral immune response. Detection and, optionally, quantitation, of immunoglobulins selectively reactive with an MRP-β epitope can be achieved through any conventional technique, such as ELISA, radioimmunoassay, Western blotting, or the like. Appropriate means of eliciting and monitoring production of antibodies with selective reactivity (binding) for other multidrug-resistance associated proteins are disclosed in Arceci et al. (1994), U.S. Patent 5,369,009, which is incorporated herein by reference. An immunoglobulin "selectively reactive with an MRP-β epitope" has binding specificity for the recognized epitope such that an antibody/epitope complex forms under conditions generally permissive of the formation of such complexes (e.g., under

conditions of time, temperature, ionic strength, pH, ionic or nonionic detergent, carrier protein, etc.). Serial dilution (titration) analysis by standard techniques is useful to estimate the avidity of antibodies in the immune serum sample for one or more epitopes unique to MRP-β. As defined herein, an "epitope unique to MRP-β" is a unique, immunogenic fragment of the full-length MRP-β polypeptide. A unique linear epitope typically ranges in size from about ten to about twenty-five amino acid residues, and frequently is about twelve to eighteen residues in length. Unique conformational epitopes also are provided herein, and comprise two or more unique fragments of the MRP-β polypeptide that, due to their juxtaposition in the folded polypeptide, form a single immunogenic epitope.

Immune serum having a high titer generally is preferred herein. Serum having a half-maximal avidity for a unique MRP-β epitope of at least about 1:1000, preferably at least about 1:10,000, can be harvested in bulk for use as a source of polyclonal antibody useful in the detection and/or quantitation of MRP-β. Polyclonal immunoglobulins can, if desired, be enriched by conventional fractionation of such serum, or can be isolated by conventional immunoadsorbent techniques, e.g., using a Protein A or Protein G chromatography resin. Immune, high titer murine or guinea pig serum alternatively is preferred herein for the production and screening of hybridomas secreting monoclonal antibodies selectively reactive with MRP-β. The present hybridomas can be produced according to well-known, standard techniques. The present monoclonal antibodies can be obtained from hybridoma culture supernatant, or from conventionally produced ascites fluid, and optionally isolated *via* immunoadsorbent chromatography or another suitable separation technique prior to use as agents to detect and/or quantitate MRP-β.

A preferred antibody, whether polyclonal or monoclonal, is selectively reactive with a unique MRP-β epitope that is displayed on the surface of MRP-β expressing cells, such as a host cell as provided herein. The preferred antibody

accordingly can be used to detect and, if desired, quantitate MRP-β expressing cells, e.g., normal or transformed cells in a mammalian body tissue or biopsy sample thereof. Exemplary analogous methods for the use of antibodies reactive with epitopes unique to P-glycoprotein are disclosed in Arceci et al. (1994), U.S. Patent 5,369,009; exemplary analogous methods for the use of antibodies reactive with epitopes unique to MRP are disclosed in Deeley et al. (1996), U.S. Patent 5,489,519. Both disclosures are incorporated herein by reference. Specifically, the preferred antibody can be used to detect MRP-β expressing cells whether such cells are host cells or mammalian body tissue cells that aberrantly express MRP-β as a result of exposure to a selection toxin such as a chemotherapeutic drug. Advantageously, intact, e.g., living, cells that display a unique MRP-β epitope can be detected by standard immunohistochemical, radiometric imaging or flow cytometry techniques. The present antibody can be used to detect and/or monitor MRP-β polypeptide production in lieu of or in addition to detecting MRP-β gene expression using the novel MRP-β nucleic acids provided herein. Thus, the antibody can be used to assess whether an aberrant phenotype, such as a multidrug-resistance phenotype, in a given cell population is associated with cell surface display of MRP-β. Further, the antibody can be used to assess the natural tissue-specific production of MRP-β, and thus to assess tissues likely to give rise to multidrug-resistant carcinomas or sarcomas. In addition, the present antibody can be used to monitor tumor biopsy samples to provide information relevant to selecting or revising a course of disease management, or to diagnosis, prognostication and/or staging of any disease associated with an abnormality affecting MRP-β. An exemplary disease is proliferative neoplastic disease. Furthermore, the present antibody can be used in a cell-sorting procedure or other cell isolation procedure to generate a substantially pure preparation of MRP-β expressing cells, or a cell population substantially depleted of MRP-β expressing cells. Each of the foregoing can be achieved through routine practice or modification of well-known techniques, including but not limited to the conjugation of a detectable

moiety (e.g., a radionuclide, fluorophore, chromophore, binding pair member, or enzyme) to the MRP-β reactive antibody.

A hybridoma secreting an MRP-β reactive monoclonal antibody of the present invention additionally provides a suitable source of nucleic acid for the routine construction of a fusion polypeptide comprising an antigen-binding fragment derived from the MRP-β reactive antibody. The present fusion polypeptide can be prepared by routine adaptation of conventional techniques therefor in Deeley et al. (1996), U.S. Patent 5,489,519 (incorporated herein by reference). The fusion polypeptide can be a truncated immunoglobulin, an immunoglobulin having a desired constant region (e.g., IgG in lieu of IgM), or a "humanized" immunoglobulin having an MRP-β reactive Fv region fused to a framework region of human origin. Additional fusion polypeptides can comprise, in addition to an MRP-β reactive antigen-binding fragment, a non-immunoglobulin polypeptide such as a cytotoxic polypeptide (e.g., diphtheria toxin, ricin) or a chemoattractant polypeptide that stimulates immune effector cells (cytotoxic T cells, natural killer cells, macrophages) to kill cells that display MRP-β. Standard techniques well-known in the art can be used to produce appropriate immunoglobulin fusion polypeptides of the present invention.

The foregoing compositions can be used for a number of purposes, including the assessment (e.g., for diagnostic purposes) of abnormalities in the structure and/or expression of a cellular MRP-β gene. Thus, for example, the invention provides a method for detecting an abornality in a cellular MRP-β gene, such as a mutation arising in germline or somatic cellular genomic DNA. Similarly, the present method provides a means for detecting chromosomal rearrangement, restriction fragment polymorphism, allelic loss or disruption of a native methylation pattern in the MRP-β gene. This method exploits the hybridization properties of an oligonucleotide probe or primer described herein. A preferred oligonucleotide is modified by the presence of a detectable label and/or a peptide nucleic acid backbone. Such oligonucleotides, which

hybridize to one or more unique fragments of a cellular MRP-β gene suspected of harboring a structural (e.g., sequence) abnormality, can be used in a diagnostic protocol as disclosed in Perry-O'Keefe et al. (1996), 93 Proc. Nat'l. Acad. Sci. USA 14670-14675, or as disclosed in Ravnik-Glavac et al. (1994), 3 Hum. Mol. Biol. 801-____. Other nucleic acid-based diagnostic methods that can be exploited for purposes of assessing MRP-β gene abnormalities are as set forth in Myers et al. (1985), 230 Science 1242; Cotton et al. (1988), 85 Proc. Nat'l. Acad. Sci. USA 4397; Suleeba et al. (1992), 217 Meth. Enzymol. 286-295; Orita et al. (1989), 86 Proc. Nat'l. Acad. Sci. USA 2766; Cotton et al. (1993), 285 Mutat. Res. 125-144; Hayashi (1992), 9 Genet. Anal. Tech. Appl. 73-79; and, Myers et al. (1985), 313 Nature 495. Additional methods are based on selective amplification and/or extension of MRP-β PCR primers, e.g., as described in Landegran et al. (1988), 241 Science 1077-1080; Nakazawa et al. (1994), 91 Proc. Nat'l. Acad. Sci. USA 360-364; and Abravaya et al. 91995), 23 Nucl. Acids Res. 675-682, and in publications referenced in Watson et al. (1992), Recombinant DNA 2nd ed., Scientific American Books and W.H. Freeman & Co., New York, NY.

Additional diagnostic and/or characterization methods using nucleic acid compositions provided herein include Northern blot, slot blot or similar methods for visualizing fluctuations, especially abnormal overproduction, in the level of cellular transcripts comprising MRP-β sequences. These methods rely on the use of MRP-β oligonucleotide probes and hybridization conditions appropriate for the formation of probe/RNA hybrids. Exemplary conditions for use with nucleic acid or modified nucleic acid probes are as set forth in Perry-O'Keefe et al. (1996), 93 Proc. Nat'l. Acad. Sci. USA 14670-14675; Current Protocols in Molecular Cloning, Ausubel et al., eds. (1989), Greene Publishing and Wiley Interscience, New York, NY and Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY. An exemplary transcript hybridization protocol is set forth in EXAMPLE 2 herein. This example confirms the association of MRP-β expression with the occurrence of a multidrug-resistance phenotype transformed cell

populations. Similar confirmation can be obtained by comparing a normal cell population with a tissue-matched transformed multidrug resistant population. Preferably, the cell populations each are derived from an exemplary mammalian body tissue, such as an epithelial tissue (e.g., mammary, respiratory tract, gastrointestinal tract, urogenital tract, paracrine, endocrine or neuroendocrine tissue). EXAMPLE 2 demonstrates that MRP-β expression is significantly elevated in multidrug-resistant derivatives of well-known cell lines, including the MCF-7 breast adenocarcinoma cell line, the HL-60 promyelocytic leukemia cell line, the A2780 ovarian carcinoma cell line, and the U937 myeloid leukemia cell line. Thus, MRP-β expression level correlates with the occurrence of multidrug-resistance rather than with derivation from a particular body tissue type.

Cellular MRP-β gene expression level similarly is expected to correlate with the maintenance or reappearance of multidrug resistance in transformed cells *in situ* following exposure to one or more chemotherapeutic drugs, or to a conventional chemosensitizer or "MDR reversal" agent. In other words, MRP-β gene expression activation or transcript stabilization is deemed likely to provide transformed cells with a selective advantage that is distinct from the advantage(s) derivable from P-glycoprotein or MRP expression. As a result, the monitoring of MRP-β transcript or polypeptide production, or gene expression level, or fluctuations therein, in one or more tumor biopsy samples is expected to provide information relevant to diagnosis, prognostication and/or staging of neoplastic disease in a cancer sufferer. Any suitable means for detecting MRP-β transcript or polypeptide production or stabilization, or gene expression level, can be applied for the present diagnostic purposes. Thus, gene expression can be monitored using any appropriate nucleic acid based method described above. MRP-β polypeptide production or accumulation can be monitored using an MRP-β antibody described herein. Any appropriate conventional method for visualizing selective binding of an antibody to its cognate epitope may be used. Appropriate

methods are described in Sambrook et al. (1989), Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

In some embodiments, diagnosis is achieved by hybridization techniques involving the use of a modified MRP-β probe as described herein. A preferred technique involved the use of a peptide-nucleic acid probe as described in Egholm et al. (1993), 365 Nature 566-568, and Perry-O'Keefe et al. (1996), 93 Proc. Natl. Acad. Sci. USA 14670-14675. Thus, for example, the protocol of EXAMPLE 2 can be routinely adapted to allow assessment of multidrug-resistant transformed cells that have survived exposure *in situ* to a chemosensitizer or to an agent that interferes with P-glycoprotein or MRP expression. Further exemplary demonstrations can be produced by routinely adapting the EXAMPLE 2 protocol to the assessment of two or more biopsy samples obtained from an individual (e.g., a cancer sufferer) at different times. Preferably, a first biopsy sample corresponds to a time of diagnosis or to a time prior to or concomitant with the onset of chemotherapy. A second biopsy sample corresponds to a timepoint at which beneficial results of chemotherapy are expected to be detectable (e.g., a time sufficiently following the onset of chemotherapy for cytotoxic effects to be observed). One or more subsequent biopsy samples may correspond to further timepoints optionally correlated with fluctuations in clinical parameters (e.g., relapse, remission, a change in disease staging, or the like). Changes (fluctuations) in MRP-β gene expression, transcript stabilization, polypeptide production, and/or polypeptide stabilization are expected to correlate with, or to predict, the emergence or attenuation of a deleterious phenotype associated with MRP-β, such as a multidrug-resistance phenotype.

It will be appreciated that the causes of multidrug-resistance phenotypes vary with each individual cell type and are not wholly accounted for by expression or overexpression of P-glycoprotein, MRP or the novel MRP-β disclosed herein. Rather, additional members of the ABC Transporter Protein family may be involved, as may be one or more members of known or novel signal tranduction pathways or intracellular metabolic or growth-regulatory pathways. The present discovery of MRP-β facilitates

investigation into the role(s) of such additional gene expression products in the acquisition and/or maintenance of a multidrug-resistance phenotype. Specifically, the discovery of MRP-β provides an improved method of identifying a gene, especially a hitherto unknown gene, expression of which contributes to emergence or maintenance of drug-resistant phenotype in transformed mammalian cells.

The identification method is an adaptation of the differential display technique disclosed in Liang et al. (1997), U.S. Patent 5,599,672 and Pardee et al. (1993), U.S. Patent 5,262,311. The method involves the steps of providing a transformed or normal cell population (the first population) derived from an exemplary mammalian tissue, such as a secretory epithelium (a nonlimiting example of which would be mammary epithelium), and culturing the cell population in the presence of a selection toxin, such that a drug-resistant derivative population (the second population) is produced. Mirsky et al. (1987), 47 Cancer Res. 2594-2598, provides an exemplary protocol for selecting a drug-resistant derivative of an immortalized human small cell lung carcinoma cell line, H69. This exemplary protocol can be adapted to use with additional cell lines, or with primary cells in culture. Thus, Hait et al. (1992), U.S. Patent 5,104,858, teaches the stepwise selection of a doxorubicin-resistant derivative of the well-known MCF-7 breast adenocarcinoma cell line. Further adaptations include, e.g., the use of a selection toxin other than adriamycin. Powell et al. (1995 and 1996), U.S. Patents 5,387,685, 5,550,149 and 5,561,141, teaches the use of bisantrene to select for a multidrug-resistant derivative of the known human ovarian carcinoma cell line, OVCAR-3 (HTB-161). If desired, the first and second populations can be selected from well-known cell lines and/or available multidrug-resistant derivatives thereof. Sunkara (1996), U.S. Patent 5,523,304, teaches the use of a multidrug-resistant human epidermoid carcinoma cell line, KBV1. Ramu et al. (1993), U.S. Patent 5,190,946, teaches the use of a murine leukemia cell line (P388) and an available multidrug-resistant derivative thereof (P388/ADR). Alternatively, the populations can be selected from biopsy samples withdrawn from an individual (e.g., a cancer sufferer) before and

after a clinical observation of multidrug resistance. Currently, the MCF-7 cell line and multidrug-resistant derivatives thereof are considered exemplary and are preferred for analysis of multidrug-resistance phenotypes.

Expressed nucleic acids (transcription products; RNA) are isolated separately from the first and second populations, and fractionated by electrophoretic resolution or another conventional technique as described in Liang et al. (1997), U.S. Patent 5,599,672. Alternatively, the expression products of the first population are used as an adsorbent to deplete the expression products of the second population of individual transcripts that are common to both populations. Thereafter, the resolved expression products are analyzed to identify one or more gene transcripts that are preferentially expressed, underexpressed or overexpressed in the second population. Such gene transcripts accordingly are associated with the multidrug-resistance phenotype. One or more probes complementary to the novel MRP-β nucleic acids disclosed herein thus can be used as an internal control to monitor successful identification of multidrug-resistance associated gene transcripts. Of course, probes complementary to nucleic acids encoding P-glycoprotein and/or MRP can be used similarly. Multidrug-resistance associated gene transcripts that are identified in this adaptation of the Liang et al. (1996) method are subjected to routine sequencing and, if previously unknown (or unknown to be correlated with multidrug-resistance) may be cloned according to conventional molecular engineering techniques as described in Current Protocols in Molecular Cloning, Ausubel et al., eds. (1989), Greene Publishing and Wiley Interscience, New York, NY. In this manner, the MRP-β probes and/or primers described herein can be used as research tools to identify and/or produce clones of hitherto unknown genes that contribute to multidrug-resistance phenotypes, such as genes that regulate cellular expression of P-glycoprotein, MRP and/or MRP-β.

The compositions provided as a result of this invention furthermore are useful tools for the characterization of MRP-β polypeptide structure, biological function and regulation in normal mammalian cells and body tissues. The availability of information

concerning the biological role of MRP-β is expected to facilitate the design, production and use of therapeutic agents to treat abnormal phenotypes, particularly disease-related phenotypes, contributed to by aberrancies in MRP-β. As part of this characterization effort, the natural expression pattern of MRP-β was surveyed in diverse mammalian body tissues. Expression products (total or poly-A(+) RNA) derived from a plurality of human body tissues were screened for hybridization with a unique MRP-β probe fragment as described in EXAMPLE 3. Transport or secretion function attributable to MRP-β was expected to affect gene expression in cells and/or tissues responsible for the secretion or excretion of cellular products or metabolites. MRP-β was observed to be expressed, at least at low (detectable baseline) levels, in substantially all body tissues. MRP-β expression similarly can be surveyed in cell types characteristic of a particular body tissue. For this more refined survey, cell types can be enriched and/or isolated from intact body tissues by convention mincing, homogenization, collagenase or trypsin digestion procedures, followed by filtration, sedimentation, adherence or panning procedures well known in the art. Alternatively, cell cultures or cell lines derived from specific body cell types may be used.

Inappropriate alteration of a cellular MRP-β gene, aberrant gene expression, transcript stabilization, or inappropriate biological function or stabilization of an MRP-β polypeptide is expected to correlate generally with tissue or cell types with a known propensity for generating transformed cells with inherent or readily acquired multidrug-resistance, especially multidrug-resistance that is refractory to treatment with known chemosensitizing agents or MDR reversal agents. MRP-β production or activity accordingly is likely to fluctuate in secretory epithelial tissues, e.g., respiratory tract, gastrointestinal tract, mammary, urogenital tract, paracrine, endocrine, and neuroendocrine tissues. Sarcomas, carcinomas, especially adenocarcinomas, originating from such tissue, particularly those originating from lung, colon, kidney, bladder, breast, ovarian, uterine, cervical, testicular, prostate or pancreatic tissue, similarly are expected to inappropriately produce MRP-β and to display or acquire multidrug-resistance

phenotypes. Indeed, confirmation of such fluctuations already has been obtained, in EXAMPLE 2.

Abnormal or aberrant phenotypes, especially multidrug-resistance associated phenotypes, that are contributed to by abnormalities affecting MRP-β, can be treated using pharmaceutical or therapeutic compositions provided herein. More specifically, the invention provides therapeutic compositions, including prophylactic, palliative and remedial compositions, useful for treatment of any disease state or deleterious condition contributed to by an abnormality affecting MRP-β. A first category of such therapeutic compositions comprise an antisense oligonucleotide, or a vector encoding an antisense oligonucleotide, that hybridizes to nucleic acid corresponding to or transcribed from a cellular MRP-β gene. Stewart et al. (1996), 51 Biochem. Pharmacol. 461-469, and Baracchini et al. (1996), U.S. Patent 5,510,239, report successful, antisense-mediated attenuation of an MRP multidrug-resistance phenotype in cultured H69AR cells: exposure to antisense oligonucleotides significantly reduced intracellular MRP transcript and polypeptide levels. The techniques and administration methods disclosed therein can be adapted to provide antisense-mediated attenuation of an MRP-β phenotype as disclosed herein. Stewart et al. (1996) report, however, that attenuation was achieved only transiently, due to the rate of cellular production of new MRP gene transcripts and/or degradation of the antisense oligonucleotide. Stewart et al. (1996) notes that, in the adriamycin selected multidrug-resistant H69AR cells, the phenotype cannot be attributed entirely to MRP expression, and for this reason counsels that antisense oligonucleotides should be used that are complementary to gene regions known to be conserved among members of the ABC Transporter Protein family. Similarly, Smyth et al. (1996), PCT Publ. WO 96/02556, reports successful, antisense oligonucleotide mediated, attenuation of a P-glycoprotein based multidrug resistance phenotype in cultured cells wherein the phenotype arises solely from P-glycoprotein production. By their nature, antisense oligonucleotides are limited to disruption of their specific target genes. Thus, the desired result of phenotypic attenuation will not be achieved where the

multidrug resistance phenotype arises from (or is preserved by) expression of one or more previously unknown genes, to which the antisense oligonucleotide is unable to hybridize effectively under intracellular conditions.

This limitation is emphasized by the disclosure herein of the present novel MRP-β. However, the present disclosure provides basis for the design and construction of the present novel antisense oligonucleotides (and oligonucleotide analogs comprising one or more of the modifications mentioned in Smyth et al. (1996)) competent to hybridize, under intracellular conditions, to all or a unique portion of the MRP-β gene or a transcript thereof. The present antisense oligonucleotides can be used alone or formulated as a cocktail together with one or more of the above-mentioned antisense oligonucleotides specific to MRP or the MDR-1 gene. Antisense oligonucleotides specific for MRP-β can be produced by conventional synthetic or biosynthetic techniques, and formulated together with pharmaceutically acceptable carriers and/or excipients into antisense pharmaceutical compositions suitable for local or systemic administration to an individual, e.g., a cancer sufferer. Suitable pharmaceutical carriers and routes of administration are described in Baracchini et al. (1996), U.S. Patent 5,510,239, and Deeley et al. (1996), U.S. Patent 5,489,519, the teachings of each of which are incorporated herein by reference.

The present MRP-β antisense oligonucleotides accordingly can be used to attenuate any undesirable phenotype associated with MRP-β, such as but not limited to a multidrug-resistance phenotype attributable in whole or in part to MRP-β expression or overexpression, e.g., in transformed cells *in situ* in mammalian body tissue. The present antisense oligonucleotides thus make possible a novel method of potentiating chemotherapy to eradicate multidrug-resistant transformed cells from the body of a mammal. The effectiveness of chemotherapy is "potentiated" (enhanced) by restoring or improving vulnerability of the transformed cells to the cytotoxic effects of a chemotherapeutic drug that otherwise would be ejected from the cell. The method

involves administering the desired chemotherapeutic drug to an individual afflicted with a multidrug-resistant transformed cell population (a tumor, e.g., a carcinoma, sarcoma, leukemia, lymphoma or lymphosarcoma), and coadministering an above-described antisense pharmaceutical composition. The administration and coadministration steps can be carried out concurrently or in any order, and can be separated by a time interval sufficient to allow uptake of either compound by the transformed cells to be eradicated. For example, the present antisense pharmaceutical composition (or a cocktail composition comprising an MRP-β antisense oligonucleotide in combination with one or more other antisense oligonucleotides) can be administered to the individual sufficiently in advance of administration of the chemotherapeutic drug to allow the antisense composition to permeate the individual's tissues, especially tissue comprising the transformed cells to be eradicated; to be internalized by transformed cells; and to disrupt MRP-β gene expression and/or protein production. The time interval required can be determined by routine pharmacokinetic means, and should be expected to vary with age, weight, sex, lean tissue content, and health status of the individual, as well as with size and body compartment location of the population of multidrug-resistant transformed cells to be eradicated.

Similar parameters should be considered in selecting a route of administration of the antisense pharmaceutical composition. Thus, the composition may be administered locally or systemically, preferably by a parenteral route. The composition can be administered intravenously, intraperitoneally, retroperitoneally, intracisternally, intramuscularly, subcutaneously, topically, intraorbitally, intranasally or by inhalation, optionally in a dispersable or controlled release excipient. One or several doses of the present composition may be administered as appropriate to achieve uptake of a sufficient amount of the present antisense oligonucleotide to produce an attenuation of multidrug-resistance phenotype in the transformed cells to be eradicated by chemotherapy.

The foregoing method alternatively can be accomplished by administration of a suitable expression vector encoding the present MRP-β antisense oligonucleotide. Use

of the present vector to internally produce or overproduce the present antisense oligonucleotide is expected to overcome the limitation noted in Stewart et al. (1996), namely, to ensure a continuous or renewable level of oligonucleotide mediated disruption of MRP-β expression or production. In this manner, the multidrug-resistance phenotype can be attenuated, if necessary, for a sufficient period of time for the coadministered chemotherapeutic agent to cause the death of transformed cells.

As noted above, all of the foregoing embodiments (MRP-β nucleic acids, host cells, MRP-β protein and antibodies thereto) are useful to characterize MRP-β biological function. Natural production of the MRP-β polypeptide also in untransformed mammalian body tissues likely endows the cell with active transport or secretion properties, by which a cell metabolite, secretion product, or biological response mediator is imported or, more likely, released from the producing cell. Thus, the normal physiological function of MRP-β may be the transport of one or more lipids, or substances comprising a moiety with lipid character, across the cell membrane. For example and without being limited by speculation, MRP-β may transport a bile acid or a steroid hormone or precursor thereof. Alternatively, MRP-β may mediate cellular uptake of short-chain fatty acids as an energy source. Thus, MRP-β may transport naturally- or synthetically-sourced substances, including chemotherapeutic drugs, that have salient physical or chemical properties in common with the natural transport substrate(s). The MRP-β expressing host cells provided herein thus are expected to facilitate investigation and characterization of substances, including cytotoxins, that are subject to MRP-β mediated transport.

Classical radioassay and/or metabolic radiolabelling techniques can be adapted routinely to screening known cell metabolites and/or secretion products to determine which may be a natural MRP-β transported substrate. Phospholipids, glycolipids, extracellular matrix precursors, endocrine hormones, proinflammatory steroids, bile acids, metabolites of any of the foregoing, and the like can be radiolabeled

by incorporation of ^{3}H, ^{35}S, ^{14}C or ^{32}P according to standard techniques. Uptake, sequestration and/or efflux of radiolabeled candidate substrates can be monitored by assessing changes in radioactivity levels (e.g., by scintillation counting, auto-radiography or a similar technique) in MRP-β host cells; in culture medium conditioned by MRP-β host cells; or, as desired, in any appropriate subcellular fraction (e.g., a scintillation fraction) prepared conventionally from MRP-β host cells. Identification of one or more natural substrates for MRP-β may be relevant to the design or selection of potential MRP-β modulators as described below.

Any conventional technique for monitoring cellular susceptibility to a cytotoxin of interest, or for monitoring intracellular accumulation, sequestration or efflux thereof, can be adapted with no more than routine experimentation to characterization of the biological (e.g., transport) properties of MRP-β. Thus, the chemosensitivity testing, accumulation and efflux assays summarized in Cole et al. (1994), 54 Cancer Res.Cancer Res. 5902-5910 can be used for characterization of MRP-β export of drugs and/or toxins such as (but not limited to) doxorubicin, vincristine, colchicine, VP-16, vinblastine, verapamil, mitoxantrone, taxol, Cyclosporin A, quinidine, progesterone, tamoxifen, epirubicin, daunorubicin, MX2, and heavy metal ions such as arsenite, arsenate, antimony tartrate, antimonate, and cadmium, whether alone or in any combination thereof. Additional suitable characterization assays include the fluorescence cell sorting techniques disclosed in Krishan (1990), 33 Meth. Cell Biol. 491-500 and in Engel et al. (1996), U.S. Patent 5,556,856 (both incorporated herein by reference), which capitalize on the fluorescent properties of daunorubicin. Another suitable assay is set forth in Zelle et al. (1996), U.S. Patent 5,543,423 (incorporated herein by reference), and is based on assessment of cellular uptake of vital dyes following a period of exposure to a potentially exportable cytotoxin. Additional published assays are summarized in Piwnica-Worms (1995), U.S. Patent 5,403,574 (incorporated herein by reference), and are based on uptake and/or efflux of fluorescent

dyes, such as rhodamine. If desired, the rapid yeast cell-growth monitoring assay set forth in Ruetz et al. (1996), 271 J. Biol. Chem. 4154-4160, also can be applied.

Additional therapeutic methods for treating abnormalities or disease states associated with MRP-β, especially with the occurrence of a multidrug-resistance phenotype, are based on the identification and use of modulators, preferably inhibitors, that affect MRP-β gene activation or expression, transcript stability, polypeptide production, post-translational processing, insertion into cellular phospholipid membranes, stabilization and/or biological function, especially transport function. A candidate substance that detectably affects (products a fluctuation in) any of the foregoing MRP-β parameters is identified herein as an MRP-β modulator. Thus, for example, a candidate that interferes with host cell resistance to a cytotoxin is identified herein as a preferred inhibitory modulator (inhibitor) of MRP-β. Candidate sustances to be subjected to screening and/or identification methods described herein available or can be produced by routine adaptations of teachings set forth in Intelligent Drug Design, A Nature Supplement, 384 Nature, Suppl. to No. 6604 (1996). Additional exemplary sources of candidate MRP-β modulators are taught in Agrafiotis et al. (1995), U.S. Patent 5,463,564; Zambias et al. (1996), PCT Publ. No. WO96/22529; Hogan et al. (1996), PCT Publ. No. WO96/12482; Hogan (1995), PCT Publ. Nos. WO95/32184 and WO95/18972; and, Beutel et al. (1995), PCT Publ. No. WO95/27072. Preferred candidate substances are small molecules, e.g., elements of a combinatorial chemistry or natural products library or pharmacopoeia. Currently, multidrug-resistant derivatives of the MCF-7 cell line, or MCF-7 host cells displaying a vector-derived, cell surface MRP-β polypeptide, are preferred herein for the identification of modulators of MRP-β. Any of the above-mentioned assays can be used for the present purpose, including high-throughput cell survival assays that monitor whether the present MRP-β expressing MCF-7 cells survive exposure to cytotoxin levels at which non-resistant cells normally succumb. For example, survival of MRP-β expressing host cells can be compared to survival of mock transfected MCF-7 cells at equivalent cytotoxin concentrations.

As mentioned previously herein, several inhibitors or antagonists of the known mammalian ABC Transporter Proteins, P-glycoprotein and MRP, have been disclosed. An inhibitor or antagonist that achieves complete interference with gene expression, polypeptide production and/or function effectively reverses the multidrug-resistance phenotype, restoring cellular vulnerability to the cytotoxic effects of an otherwise exported chemotherapeutic drug. An inhibitor or antagonist that achieves partial interference also can be considered beneficial clinically, in that partial interference with drug export function "attenuates" or reduces penetrance of the multidrug resistance phenotype. Upon treatment with a partial inhibitor, cellular vulnerability to cytotoxins is increased, albeit not fully restored. Such substances are commonly referred to in the art as "MDR reversal agents" or "chemosensitizing agents." Powell et al. (1996), U.S. Patents 5,561,141 and 5,550,149; Powell et al. (1995), U.S. Patent 5,387,685; Engel et al. (1996), U.S. Patent 5,556,856; Zelle et al. (1996), U.S. Patent 5,543,423; Sunkara (1996), U.S. Patent 5,523,304; Sunkara et al. (1993), U.S. Patents 5,190,957 and 5,182,293; Sarkadi et al. (1995), PCT Publ. WO 95/31474; Piwnica-Worms (1995), U.S. Patent 5,403,574; Hait et al. (1992), U.S. Patent 5,104,856. Little structural similarity has been observed between the known classes of MDR reversal agents, or between reversal agents and exported cytotoxic drugs. Thus, high through-put screening, e.g., of naturally-sourced or synthetic chemicals in a pharmacopoeia or combinatorial library, was required to identify each currently known MDR reversal agent. Furthermore, the majority of known MDR reversal agents are specific inhibitors of either P-glycoprotein or of MRP: little to no cross-inhibition has been observed. Thus, it is expected that empirical screening will be required, for the identification of one or more modulators, preferably inhibitors, of MRP-β. Exemplary identification or screening protocols are referenced herein and appear herein in EXAMPLES 4 and 5.

All modulators of MRP-β, including partial modulators, that are identified through practice of the above-described methods, or routine modifications thereof, are

considered to be within the scope of the present invention. Small molecule modulators are preferred. Inhibitory modulators (inhibitors) are especially contemplated herein. For therapeutic administration purposes, a modulator of the present invention can be administered to an individual as a pharmaceutically acceptable salt or derivative. Further, the present modulator can be formulated with any pharmaceutically acceptable carrier, excipient, adjuvant or vehicle. Appropriate pharmaceutically acceptable salts, derivatives, carriers, excipients, adjuvants and vehicles are as disclosed in Zelle et al. (1996), U.S. Patent 5,543,423 (which is incorporated herein by reference) or can be produced or selected by routine modifications thereof.

The present MRP-β modulator accordingly can be used to mitigate severity of, up to an including to abrogate, any phenotype associated with an abnormality affecting MRP-β. That is, the present modulator may be used to treat or palliate any disease or condition affecting the health status of an individual, such as a human, that arises from the MRP-β abnormality. The modulator also may be administered prophylactically, to avert or delay the onset of a deleterious phenotype associated with MRP-β dysfunction. In particular, the present MRP-β modulator is useful to attenuate a multidrug-resistance phenotype attributable in whole or in part to MRP-β gene abnormality, gene expression, transcript stabilization, or polypeptide production, processing, stability or biological function, e.g., in transformed cells *in situ* in mammalian body tissue. Preferred inhibitory modulators make possible novel methods, for example, of potentiating chemotherapy to eradicate multidrug-resistant transformed cells from the body of a mammal. As with the antisense pharmaceutical composition method discussed herein, the effectiveness of chemotherapy is enhanced by restoring or improving vulnerability of the transformed cells to the cytotoxic effects of a chemotherapeutic drug that otherwise would be ejected from the cell. The present, modulator-based method involves administering the modulator alone or as an adjuvant to the desired chemotherapeutic drug, to an individual afflicted with a multidrug-resistant tumor, e.g., a carcinoma, sarcoma, leukemia, lymphoma or lymphosarcoma.

The chemotherapeutic drug and MRP-β modulator may be administered concurrently or in any order, and can be separated by a time interval sufficient to allow uptake of either compound by the transformed cells to be eradicated. If desired, the present modulator can be administered alone or in a cocktail, combined with one or more known MDR reversal agents (e.g., agents that affect MRP or P-glycoprotein).

Preferably, the modulator is administered to the individual sufficiently in advance of administration of the chemotherapeutic drug to allow the modulator to permeate the individual's tissues, especially tissue comprising the transformed cells to be eradicated; to be internalized by transformed cells; and to impair MRP-β mediated cytotoxin sequestration or efflux. The time interval required can be determined by routine pharmacokinetic means, and should be expected to vary with age, weight, sex, lean tissue content, and health status of the individual, as well as with size and body compartment location of the population of multidrug-resistant transformed cells to be eradicated. Similar parameters should be considered in selecting a route of administration of the modulator. Thus, the modulator may be administered locally or systemically, preferably by a parenteral route. It can be administered intravenously, intraperitoneally, retroperitoneally, intracisternally, intramuscularly, subcutaneously, topically, intraorbitally, intranasally or by inhalation, optionally in a dispersable or controlled release excipient. One or several doses may be administered as appropriate to achieve uptake of a sufficient amount of the present modulator to produce an attenuation of multidrug-resistance phenotype in the transformed cells to be eradicated by chemotherapy. As a result of therapeutic intervention with an MRP-β modulator (preferably, an inhibitory modulator), penetrance of an abnormal or deleterious phenotype (generally, but not limited to, a multidrug resistance phenotype) is attentuated, even abrogated, in the treated individual. The overall dosage and administration protocol for treatment with the present modulator may be designed and optimized by the clinical practitioner through the application of routine clinical skill.

Practice of the invention will be still more fully understood from the following EXAMPLES, which are presented solely to illustrate principles and operation of the invention, and should not be construed as limiting scope of the invention in any way.

Example 1: Isolation and cloning of full-length MRP-β cDNA.

A unique fragment (SEQ ID No: 3) of the novel MRP-β gene was identified by computer-assisted screening of a nucleic acid database corresponding to a human endothelial cell expression library. The library was prepared using cellular RNA transcripts produced in human microvascular endothelial cells (HUMVEC) isolated from breast tissue and maintained in primary culture in the presence of a commercially available extracellular matrix composition (Matrigel), and in the presence of appropriate growth and differentiation factors (e.g., vascular endothelial cell growth factor (VEGF)). These conditions had previously been shown to preserve cell viability and substantially differentiated phenotype *in vitro*.

A nucleic acid probe corresponding to the SEQ ID No: 3 unique fragment was prepared by conventional techniques. This probe was used for hybridization screening of the HUMVEC expression library for the presence of MRP-β cDNAs. This procedure yielded an MRP-β cDNA (residues 67-4847 FIGURE 1 and SEQ ID No: 1), 4.78 kb (kilobases) in length. The clone comprising this cDNA insert has been designated fohd013a05m and deposited with the American Type Culture Collection. Two independent cDNA clones comprising approximately 60 residues upstream (5') from the fohd013a05m MRP-β insert were isolated by hybridization screening of human brain and liver cDNA libraries with a nucleic acid probe corresponding approximately to the 5' 0.5 kb of the fohd013a05m MRP-β insert. This probe was prepared by isolating an approximately 0.5 kb SacI fragment from fohd013a05m. The cDNA sequence presented in SEQ ID No: 1 comprises the sequence of the fohd013a05m MRP-β insert and the sequence of an additional 66 upstream (5') nucleotides. The open reading frame (ORF)

of the SEQ ID No: 1 cDNA encodes an MRP-β polypeptide (SEQ ID No: 2) 1437 amino acid residues in length and in addition, includes a 0.42 kb 3' untranslated region. The ORF start site indicated in SEQ ID No: 1 (at nucleotides 116-118 of SEQ ID No: 1) is the first in-frame ATG codon downstream from the TGA stop codon at nucleotides 23-25 of SEQ ID No: 1.

Example 2: Correlation of MRP-β expression level with multidrug-resistance phenotype.

Involvement of the present novel MRP-β gene in the acquisition or maintenance of a multidrug-resistance phenotype has been confirmed by comparing the level of MRP-β gene expression in immortalized, transformed cells (wild-type or parent cells) that have not acquired the property of multidrug-resistance with the level in a multidrug-resistant derivative of the parent cell population. One set of exemplary parent and multidrug-resistant derivative cell lines are described in Mirsky et al. (1987), 47 Cancer Res. 2594-2598 (parent and multidrug-resistant (MDR) derivative of the H69 human small cell lung carcinoma line). Additional exemplary parent and multidrug-resistant derivative lines are described in Slapak et al. (1994), 84 Blood 3113-3121 (parent and MDR derivative of the U937 human myeloid leukemia line); Batist et al. (1986), 261 J. Biol. Chem. 15544-15549 (parent and MDR derivative of the MCF-7 human breast adenocarcimoma line); March et al. (1986), 46 Cancer Res. 4053-4057 (parent and MDR derivative of the HL-60 human promyelocytic leukemia line); and, Hamilton et al. (1984), 11 Sem. Oncol. 285-298 (parent and MDR derivative of the A2780 human ovarian carcinoma line). Each of the foregoing references is incorporated herein by reference. To demonstrate correlation between MRP-β gene expression and multidrug-resistance phenotype, parental (wild-type) and adriamycin-selected multidrug resistant MCF-7 cells were cultured to confluency under standard cell culture conditions and treated to release expressed nucleic acid transcripts, which were subjected to Northern blot analysis.

Preparation of cellular RNA. Expressed nucleic acids were isolated from the exemplary parental and resistant MCF-7 cells using components of the Qiagen, Inc. RNeasy Total RNA kit, generally as in the Qiagen, Inc. RNeasy Handbook (1995). Kit components include spin columns, collection tubes, lysis buffer, wash buffer and RNAse free water. Expressed nucleic acid extracts were prepared by suspending cells in lysis buffer supplemented with 2-mercaptoethanol and passage of the resulting mixture through a Qiagen, Inc. Qiashredder homogenization column. RNA was purified from the resulting lysate using a Qiagen, Inc. RNeasy column supplied with the kit. The lysate was loaded onto the RNeasy colum, washed and RNA was eluted generally as described in the RNeasy Handbook.

Electrophoretic Resolution of Expressed RNAs. Agarose-formaldehyde slab gels (1.0-2.5% agarose) were prepared and cast according to standard techniques. RNA samples (10-30 μg total RNA or 1-3 μg PolyA(+) RNA) were combined with denaturing bromophenol blue sample buffer, loaded onto the gel and subjected to electrophoresis by passage of 100 volts through the gel chamber for about 3 hours or until the bromophenol blue dye front had migrated about 10 cm into the gel. A photograph of the resolved gel was obtained prior to transfer of resolved RNAs to nylon.

Replica Transfer of Resolved RNAs to Nylon. The gel comprising resolved cellular MRP-β transcript was prepared for transfer by soaking in 0.05 N NaOH, 0.15 M NaCl for 20-30 minutes, followed by neutralization in 0.1 M Tris pH 7.5, 0.15 M NaCl for 30 minutes. RNA contents of the neutralized gel were then transferred to a nylon membrane using a Posiblot apparatus (Stratagene, Inc.). Transfer was allowed to proceed for 1 hour, following which the transferred, resolved RNAs were crosslinked to the membrane using UV light generated by a Stratalinker apparatus (Stratagene, Inc.). The location of resolved RNAs on the membrane was visualized by staining with methylene blue. The positions of the RNA ladder, 18S, and 28S ribosomal RNAs were marked on a photograph taken of the stained membrane, which was then destained according to standard procedure.

Preparation of detectably labeled MRP-β Probe. A unique fragment (e.g., SEQ ID No: 3) of the MRP-β cDNA was used for the preparation of a radiolabeled hybridization probe for visualizing the electrophoretically resolved, full-length MRP-β transcript expressed in parent (wild-type) and MDR MCF-7 cells. The probe was prepared using the Stratagene, Inc. Prime It-RmT Primer Labeling Kit, generally according to the protocol supplied by the manufacturer (see also Feinberg et al. (1984) 137 Anal. Biochem. 266-267 and Feinberg et al. (1983), 132 Anal. Biochem. 6-13). Kit components include control DNA, Magenta thermostable DNA polymerase, stop mix, and dehydrated single-use reaction mixtures comprising random primers, nucleotides, buffer and cofactors required by the polymerase. To prepare the probe, 50 ng MRP-β DNA (e.g., cDNA insert comprising SEQ ID No: 3) in aqueous solution was added to a kit single-use reaction mixture and boiled to ensure denaturation. To obtain incorporation of at least 10^6 cpm/μL, 5 μL [α-^{32}P]dCTP (6000 Ci/mmol) was added to the mixture, followed by 3 μL Magenta polymerase (4U/uL). Probe synthesis was conducted at 37 °C for 10 minutes, then stopped by the addition of 2 μL stop mix. To reduce background, the labeled probe was purified using a chromaspin TE-10 column prior to use for hybridization.

Hybridization. Prior to contact with the radiolabeled probe, the nylon membrane comprising crosslinked, electrophoretically resolved MCF-7 cellular transcripts was prehybridized for 20 minutes at 65 °C in 10 mL Rapid-hyb solution available from Amersham, Inc. The prepared probe was boiled for 5 minutes to ensure denaturation, and added to an additional 10 mL Rapid-Hyb solution. The prehybridization solution was exchanged for probe solution, and the probe was allowed to hybridize to membrane-bound transcripts for 2 hours at 65 °C. Excess, unhybridized probe was removed by washing the membrane in 2X SSC, 0.1 % SDS for 20 minutes, either at room temperature or at 42 °C. Thereafter, the membrane was washed in 0.1X SSC, 0.1% SDS for 20 min. at 65 °C. The 65 °C wash step was repeated if necessary to obtain a satisfactory signal-to-background ratio as assessed by geiger counter. Results

were visualized by exposure to X-ray film according to standard procedures. Thereafter, MRP-β probe was stripped by addition of a boiling solution of 0.5% (w/v) SDS (0.1X SSC, 0.1% SDS also can be used as a stripping solution). Significance of the MRP-β results were verified by rehybridization of the membrane with a probe specific for the transcript of a conventionally used housekeeping or structural gene (e.g., Ef-TU or actin).

Results. A single 6 kb transcript was visualized by the MRP-β probe in both wild-type and MDR MCF-7 cellular RNA. A significantly elevated level of the MRP-β transcript was observed in the MDR derivative cell line, which is reported in Batist et al. (1986) to be 192-fold more resistant to adriamycin than the parental (wild-type) MCF-7 human breast adenocarcinoma cell line. Consistent results showing elevated levels of MRP-β gene expression were observed in comparison studies of parental and MDR derivative cell lines established from human ovarian carcinoma (A2780; Hamilton et al. (1984)) and human leukemias (HL-60; March et al. (1986), and U937; Slapak et al. (1994)). Thus, MRP-β gene expression level correlates with the acquisition of a multidrug-resistance phenotype, rather than with the body tissue type in which a particular tumor arises.

Example 3: Expression of MRP-β in Mammalian Body Tissues.

As noted above, a clearly detectable baseline level of MRP-β gene expression was observed even in wild-type tumor cell lines. To establish whether this baseline expression correlates with tumorigenesis, the above-described radiolabeled MRP-β probe was hybridized to commercially available human multiple tissue Northern (MTN) blots (Clontech, Inc.), generally according to the manufacturer's directions and the procedure described above in EXAMPLE 2. Tissues from which polyA(+)RNA was analyzed included heart, brain, placenta, lung, liver, skeletal muscle, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, colon (mucosal lining) and peripheral blood leukocyte.

Results. Clearly detectable baseline expression of a 6 kb MRP-β transcript was observed in substantially all normal human body tissues surveyed, with the highest expression level being observed in heart tissue. The survey samples represent expressed RNAs isolated from lysates of whole tissue, rather than from specific cell types characteristic of one or more body tissues. Taken together with the isolation of MRP-β cDNAs from a HUMVEC expression library (described in EXAMPLE 1), the present MTN survey data is consistent with substantially ubiquitous baseline expression of MRP-β in vasculature or microvasculature.

Example 4: Confirmation that MRP-β expression is sufficient to confer a survival advantage on cells exposed to a cytotoxic agent.

Host cells stably transfected with an MRP-β expression vector as described herein are expected to gain a significant survival advantage, relative to source (untransfected) or control (antisense transfected) cells. To establish this survival advantage, triplicate cultures of MRP-β host cells, control cells and source cells (e.g., MCF-7 human breast adenocarcinoma cells) are generated in 24-well cell culture plates. Once the cultures have attained at least 80% confluency, lethal or sub-lethal amounts of a cytotoxin (e.g., adriamycin, bisantrene) are added to each well. After a sufficient period of time for cytotoxic effects to be manifested (e.g., 16-24 hours in culture), culture media comprising the cytotoxic drug are aspirated or otherwise removed, and cells are stained with a vital dye such as Trypan blue. Which commercially available vital dye is used in this procedure is a matter of choice; thus, sulforodoamine B (see Powell et al. (1990), U.S. Patent 5,550,149) could be used in lieu of Trypan blue. The number of cells that remain viable (e.g., capable of excluding the dye) are counted using a hemocytometer, flow cytometer or other appropriate device.

Expected Results. MRP-β expressing host cells are expected to acquire the capability of surviving exposure to otherwise lethal amounts of a cytotoxin, such as adriamycin or bisantrene. Analysis of the differential between toxin levels that are lethal

to source or control cells, and that which is lethal to MRP-β host cells, is expected to provide a predictive index of the recalcitrance of MRP-β expressing transformed cells *in situ* to chemotherapy. Repetition of this cytotoxicity assay with additional toxins (e.g., environmentally or occupationally derived toxins, metabolites or chemotherapeutic drugs) is expected to elucidate the nature of substances exportable or sequestrable by MRP-β and to uncover specific differences between the characteristics of substrates transported by MRP-β and those transported by known ATP Transporter Protein superfamily members such as P-glycoprotein and/or MRP.

Screening for a modulator of MRP-β. The present cytotoxicity assay can be adapted routinely to provide a rapid assay for screening candidate modulators of MRP-β. In this adaptation, host cell cultures are incubated in the presence of a toxin to which MRP-β expression confers a survival advantage. The level of toxin exposure is sub-lethal to host cells but lethal to source cells or control cells. Candidate MRP-β modulators (e.g., inhibitors) are added to the cell cultures, which are incubated for a sufficiently further period of time for cytotoxicity to be manifested (e.g., 16-24 hours). A candidate that attenuates or abrogates the host cells' survival advantage is identified as an MRP-β inhibitor. Guidelines for this adaptation of the present cytotoxicity assay may be found in Powell et al. (1996), U.S. Patent 5,550,149. Candidate MRP-β modulators may be selected from any appropriate source, such as a pharmacopeia of natural or synthetic substances, combinatorial chemistry library, phage display epitope library, or the like. Appropriate sources are available or can be produced by routine adaptations of teachings set forth in Intelligent Drug Design, A Nature Supplement, 384 Nature, Suppl. to No. 6604 (1996). Additional exemplary sources of candidate MRP-β modulators are taught in Agrafiotis et al. (1995), U.S. Patent 5,463,564; Zambias et al. (1996), PCT Publ. No. WO96/22529; Hogan et al. (1996), PCT Publ. No. WO96/12482; Hogan (1995), PCT Publ. Nos. WO95/32184 and WO95/18972; and, Beutel et al. (1995), PCT Publ. No. WO95/27072.

Example 5: Assessment of MRP-β Mediated Drug Efflux.

Without being limited by speculation, it is likely that MRP-β confers the above-described survival advantage by mediating sequestration or efflux of one or more cytotoxins. That is, it is likely that MRP-β is a member of the ABC Transporter Protein superfamily that carries out an export function. However, routine empirical testing is required to confirm whether MRP-β exports one or more toxic substances, or imports one or more nutrients or energy sources, such as sugars or fatty acids of dietary or other metabolic origin. A number of conventional protocols can be practiced, with such routine modifications as may be deemed appropriate by the practitioner, to establish whether MRP-β mediates toxin export. A presently preferred technique capitalizes on the fluorescent properties of anthracycline toxins (including adriamycin (doxorubicin) and daunamycin), such that toxin accumulation and/or efflux from MRP-β expressing host cells can be monitored by fluorescence histochemistry or, preferably, by fluorescence-activated flow cytometry. An example of this technique is described in Krishan (1990), 33 Meth. Cell Biol. 491-500, incorporated herein by reference.

Fluorescent labeling. Viable MRP-β host cells (at least 10,000) are suspended in culture medium in the sampling cuvette of a flow cytometer, such as the EPICS 753 apparatus (Coulter Electronics, Inc.) equipped with an argon laser for fluorophore excitation at 488 nm, and a photomultiplier (e.g., MDADS II data acquisition apparatus) for detection of 530 nm emissions. The cuvette is maintained at 37 °C, and adriamycin or daunomycin are added to a final concentration of 1-3 μM prior to cell sorting. Two-parameter histograms are generated based on cellular fluorescence and incubation time (typically 30 to 60 minutes) in the presence of the fluorescent toxin.

Expected results. MRP-β host cells are expected to internalize and/or retain significantly lower levels of adriamycin or daunomycin than source cells or control cells.

Screening for a modulator of MRP-β. The above drug efflux assay can be adapted routinely to provide a rapid assay for screening candidate modulators of MRP-β.

In this adaptation, a candidate MRP-β modulator is added to the cuvette during the fluorophore uptake incubation. A candidate that attenuates or abrogates the host cells' capacity for fluorophore efflux is identified as an MRP-β inhibitor. Guidelines for this adaptation may be found in Krishan (1990), 33 Meth. Cell Biol. 491-500). Candidate MRP-β modulators may be selected from any appropriate source, such as the sources mentioned in EXAMPLE 4.

Equivalents

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. For example, the invention may be embodied in one or more variants, e.g., deletion, addition or substitution variants, of the nucleic acid and/or protein sequences disclosed herein, such as may be produced routinely by mutagenesis or other conventional molecular engineering and biosynthetic production techniques. Specifically, the invention may be embodied in any variant, whether biosynthetically produced or isolated from a natural source, the expression or overexpression of which endows a mammalian cell with a multidrug-resistance phenotype. More specifically, the invention may be embodied in a variant which, when expressed or overexpressed, endows a mammalian cell with resistance to the cytotoxic effects of MRP-β transportable drugs. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes which come within the meaning and range of equivalency of the claims are intended to be embraced therein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: SHYJAN, Andrew

(ii) TITLE OF INVENTION: NOVEL MULTIDRUG RESISTANCE-ASSOCIATED POLYPEPTIDE

(iii) NUMBER OF SEQUENCES: 8

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Testa, Hurwitz & Thibeault
(B) STREET: 125 High St.
(C) CITY: Boston
(D) STATE: MA
(E) COUNTRY: USA
(F) ZIP: 02110

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: WALLER, Patrick R H
(B) REGISTRATION NUMBER: 41,418
(C) REFERENCE/DOCKET NUMBER: MIL-001CP

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (617) 248-7000
(B) TELEFAX: (617) 248-7100

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4847 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 116..4426

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
GGCTCATGCT CGGGAGCGTG GTTGAGCGGC TGGCGCGGTT GTCCTGGAGC AGGGGCGCAG      60

GAATTCTGAT GTGAAACTAA CAGTCTGTGA GCCCTGGAAC CTCCACTCAG AGAAG ATG      118
                                                             Met
                                                               1

AAG GAT ATC GAC ATA GGA AAA GAG TAT ATC ATC CCC AGT CCT GGG TAT      166
Lys Asp Ile Asp Ile Gly Lys Glu Tyr Ile Ile Pro Ser Pro Gly Tyr
                5                  10                  15

AGA AGT GTG AGG GAG AGA ACC AGC ACT TCT GGG ACG CAC AGA GAC CGT      214
Arg Ser Val Arg Glu Arg Thr Ser Thr Ser Gly Thr His Arg Asp Arg
        20                  25                  30

GAA GAT TCC AAG TTC AGG AGA ACT CGA CCG TTG GAA TGC CAA GAT GCC      262
Glu Asp Ser Lys Phe Arg Arg Thr Arg Pro Leu Glu Cys Gln Asp Ala
    35                  40                  45

TTG GAA ACA GCA GCC CGA GCC GAG GGC CTC TCT CTT GAT GCC TCC ATG      310
Leu Glu Thr Ala Ala Arg Ala Glu Gly Leu Ser Leu Asp Ala Ser Met
 50                  55                  60                  65

CAT TCT CAG CTC AGA ATC CTG GAT GAG GAG CAT CCC AAG GGA AAG TAC      358
His Ser Gln Leu Arg Ile Leu Asp Glu Glu His Pro Lys Gly Lys Tyr
                 70                  75                  80

CAT CAT GGC TTG AGT GCT CTG AAG CCC ATC CGG ACT ACT TGC AAA CAC      406
His His Gly Leu Ser Ala Leu Lys Pro Ile Arg Thr Thr Cys Lys His
            85                  90                  95

CAG CAC CCA GTG GAC AAT GCT GGG CTT TTT TCC TGT ATG ACT TTT TCG      454
Gln His Pro Val Asp Asn Ala Gly Leu Phe Ser Cys Met Thr Phe Ser
        100                 105                 110

TGG CTT TCT TCT CTG GCC CGT GTG GCC CAC AAG AAG GGG GAG CTC TCA      502
Trp Leu Ser Ser Leu Ala Arg Val Ala His Lys Lys Gly Glu Leu Ser
    115                 120                 125

ATG GAA GAC GTG TGG TCT CTG TCC AAG CAC GAG TCT TCT GAC GTG AAC      550
Met Glu Asp Val Trp Ser Leu Ser Lys His Glu Ser Ser Asp Val Asn
130                 135                 140                 145

TGC AGA AGA CTA GAG AGA CTG TGG CAA GAA GAG CTG AAT GAA GTT GGG      598
Cys Arg Arg Leu Glu Arg Leu Trp Gln Glu Glu Leu Asn Glu Val Gly
                150                 155                 160

CCA GAC GCT GCT TCC CTG CGA AGG GTT GTG TGG ATC TTC TGC CGC ACC      646
Pro Asp Ala Ala Ser Leu Arg Arg Val Val Trp Ile Phe Cys Arg Thr
            165                 170                 175

AGG CTC ATC CTG TCC ATC GTG TGC CTG ATG ATC ACG CAG CTG GCT GGC      694
Arg Leu Ile Leu Ser Ile Val Cys Leu Met Ile Thr Gln Leu Ala Gly
        180                 185                 190

TTC AGT GGA CCA GCC TTC ATG GTG AAA CAC CTC TTG GAG TAT ACC CAG      742
Phe Ser Gly Pro Ala Phe Met Val Lys His Leu Leu Glu Tyr Thr Gln
    195                 200                 205
```

GCA ACA GAG TCT AAC CTG CAG TAC AGC TTG TTG TTA GTG CTG GGC CTC 790
Ala Thr Glu Ser Asn Leu Gln Tyr Ser Leu Leu Leu Val Leu Gly Leu
210 215 220 225

CTC CTG ACG GAA ATC GTG CGG TCT TGG TCG CTT GCA CTG ACT TGG GCA 838
Leu Leu Thr Glu Ile Val Arg Ser Trp Ser Leu Ala Leu Thr Trp Ala
230 235 240

TTG AAT TAC CGA ACC GGT GTC CGC TTG CGG GGG GCC ATC CTA ACC ATG 886
Leu Asn Tyr Arg Thr Gly Val Arg Leu Arg Gly Ala Ile Leu Thr Met
245 250 255

GCA TTT AAG AAG ATC CTT AAG TTA AAG AAC ATT AAA GAG AAA TCC CTG 934
Ala Phe Lys Lys Ile Leu Lys Leu Lys Asn Ile Lys Glu Lys Ser Leu
260 265 270

GGT GAG CTC ATC AAC ATT TGC TCC AAC GAT GGG CAG AGA ATG TTT GAG 982
Gly Glu Leu Ile Asn Ile Cys Ser Asn Asp Gly Gln Arg Met Phe Glu
275 280 285

GCA GCA GCC GTT GGC AGC CTG CTG GCT GGA GGA CCC GTT GTT GCC ATC 1030
Ala Ala Ala Val Gly Ser Leu Leu Ala Gly Gly Pro Val Val Ala Ile
290 295 300 305

TTA GGC ATG ATT TAT AAT GTA ATT ATT CTG GGA CCA ACA GGC TTC CTG 1078
Leu Gly Met Ile Tyr Asn Val Ile Ile Leu Gly Pro Thr Gly Phe Leu
310 315 320

GGA TCA GCT GTT TTT ATC CTC TTT TAC CCA GCA ATG ATG TTT GCA TCA 1126
Gly Ser Ala Val Phe Ile Leu Phe Tyr Pro Ala Met Met Phe Ala Ser
325 330 335

CGG CTC ACA GCA TAT TTC AGG AGA AAA TGC GTG GCC GCC ACG GAT GAA 1174
Arg Leu Thr Ala Tyr Phe Arg Arg Lys Cys Val Ala Ala Thr Asp Glu
340 345 350

CGT GTC CAG AAG ATG AAT GAA GTT CTT ACT TAC ATT AAA TTT ATC AAA 1222
Arg Val Gln Lys Met Asn Glu Val Leu Thr Tyr Ile Lys Phe Ile Lys
355 360 365

ATG TAT GCC TGG GTC AAA GCA TTT TCT CAG AGT GTT CAG AAA ATC CGC 1270
Met Tyr Ala Trp Val Lys Ala Phe Ser Gln Ser Val Gln Lys Ile Arg
370 375 380 385

GAG GAG GAG CGT CGG ATA TTG GAA AAA GCC GGG TAC TTC CAG AGC ATC 1318
Glu Glu Glu Arg Arg Ile Leu Glu Lys Ala Gly Tyr Phe Gln Ser Ile
390 395 400

ACT GTG GGT GTG GCT CCC ATT GTG GTG GTG ATT GCC AGC GTG GTG ACC 1366
Thr Val Gly Val Ala Pro Ile Val Val Val Ile Ala Ser Val Val Thr
405 410 415

TTC TCT GTT CAT ATG ACC CTG GGC TTC GAT CTG ACA GCA GCA CAG GCT 1414
Phe Ser Val His Met Thr Leu Gly Phe Asp Leu Thr Ala Ala Gln Ala
420 425 430

TTC ACA GTG GTG ACA GTC TTC AAT TCC ATG ACT TTT GCT TTG AAA GTA 1462
Phe Thr Val Val Thr Val Phe Asn Ser Met Thr Phe Ala Leu Lys Val
435 440 445

ACA CCG TTT TCA GTA AAG TCC CTC TCA GAA GCC TCA GTG GCT GTT GAC 1510
Thr Pro Phe Ser Val Lys Ser Leu Ser Glu Ala Ser Val Ala Val Asp
450 455 460 465

AGA TTT AAG AGT TTG TTT CTA ATG GAA GAG GTT CAC ATG ATA AAG AAC 1558
Arg Phe Lys Ser Leu Phe Leu Met Glu Glu Val His Met Ile Lys Asn
470 475 480

AAA CCA GCC AGT CCT CAC ATC AAG ATA GAG ATG AAA AAT GCC ACC TTG 1606
Lys Pro Ala Ser Pro His Ile Lys Ile Glu Met Lys Asn Ala Thr Leu
485 490 495

GCA TGG GAC TCC TCC CAC TCC AGT ATC CAG AAC TCG CCC AAG CTG ACC 1654
Ala Trp Asp Ser Ser His Ser Ser Ile Gln Asn Ser Pro Lys Leu Thr
500 505 510

CCC AAA ATG AAA AAA GAC AAG AGG GCT TCC AGG GGC AAG AAA GAG AAG 1702
Pro Lys Met Lys Lys Asp Lys Arg Ala Ser Arg Gly Lys Lys Glu Lys
515 520 525

GTG AGG CAG CTG CAG CGC ACT GAG CAT CAG GCG GTG CTG GCA GAG CAG 1750
Val Arg Gln Leu Gln Arg Thr Glu His Gln Ala Val Leu Ala Glu Gln
530 535 540 545

AAA GGC CAC CTC CTC CTG GAC AGT GAC GAG CGG CCC AGT CCC GAA GAG 1798
Lys Gly His Leu Leu Leu Asp Ser Asp Glu Arg Pro Ser Pro Glu Glu
550 555 560

GAA GAA GGC AAG CAC ATC CAC CTG GGC CAC CTG CGC TTA CAG AGG ACA 1846
Glu Glu Gly Lys His Ile His Leu Gly His Leu Arg Leu Gln Arg Thr
565 570 575

CTG CAC AGC ATC GAT CTG GAG ATC CAA GAG GGT AAA CTG GTT GGA ATC 1894
Leu His Ser Ile Asp Leu Glu Ile Gln Glu Gly Lys Leu Val Gly Ile
580 585 590

TGC GGC AGT GTG GGA AGT GGA AAA ACC TCT CTC ATT TCA GCC ATT TTA 1942
Cys Gly Ser Val Gly Ser Gly Lys Thr Ser Leu Ile Ser Ala Ile Leu
595 600 605

GGC CAG ATG ACG CTT CTA GAG GGC AGC ATT GCA ATC AGT GGA ACC TTC 1990
Gly Gln Met Thr Leu Leu Glu Gly Ser Ile Ala Ile Ser Gly Thr Phe
610 615 620 625

GCT TAT GTG GCC CAG CAG GCC TGG ATC CTC AAT GCT ACT CTG AGA GAC 2038
Ala Tyr Val Ala Gln Gln Ala Trp Ile Leu Asn Ala Thr Leu Arg Asp
630 635 640

AAC ATC CTG TTT GGG AAG GAA TAT GAT GAA GAA AGA TAC AAC TCT GTG 2086
Asn Ile Leu Phe Gly Lys Glu Tyr Asp Glu Glu Arg Tyr Asn Ser Val
645 650 655

CTG AAC AGC TGC TGC CTG AGG CCT GAC CTG GCC ATT CTT CCC AGC AGC 2134
Leu Asn Ser Cys Cys Leu Arg Pro Asp Leu Ala Ile Leu Pro Ser Ser
660 665 670

GAC CTG ACG GAG ATT GGA GAG CGA GGA GCC AAC CTG AGC GGT GGG CAG 2182
Asp Leu Thr Glu Ile Gly Glu Arg Gly Ala Asn Leu Ser Gly Gly Gln
675 680 685

CGC CAG AGG ATC AGC CTT GCC CGG GCC TTG TAT AGT GAC AGG AGC ATC 2230
Arg Gln Arg Ile Ser Leu Ala Arg Ala Leu Tyr Ser Asp Arg Ser Ile
690 695 700 705

TAC ATC CTG GAC GAC CCC CTC AGT GCC TTA GAT GCC CAT GTG GGC AAC 2278
Tyr Ile Leu Asp Asp Pro Leu Ser Ala Leu Asp Ala His Val Gly Asn
710 715 720

CAC ATC TTC AAT AGT GCT ATC CGG AAA CAT CTC AAG TCC AAG ACA GTT 2326
His Ile Phe Asn Ser Ala Ile Arg Lys His Leu Lys Ser Lys Thr Val
725 730 735

CTG TTT GTT ACC CAC CAG TTA CAG TAC CTG GTT GAC TGT GAT GAA GTG 2374
Leu Phe Val Thr His Gln Leu Gln Tyr Leu Val Asp Cys Asp Glu Val
740 745 750

ATC TTC ATG AAA GAG GGC TGT ATT ACG GAA AGA GGC ACC CAT GAG GAA 2422
Ile Phe Met Lys Glu Gly Cys Ile Thr Glu Arg Gly Thr His Glu Glu
755 760 765

CTG ATG AAT TTA AAT GGT GAC TAT GCT ACC ATT TTT AAT AAC CTG TTG 2470
Leu Met Asn Leu Asn Gly Asp Tyr Ala Thr Ile Phe Asn Asn Leu Leu
770 775 780 785

CTG GGA GAG ACA CCG CCA GTT GAG ATC AAT TCA AAA AAG GAA ACC AGT 2518
Leu Gly Glu Thr Pro Pro Val Glu Ile Asn Ser Lys Lys Glu Thr Ser
790 795 800

GGT TCA CAG AAG AAG TCA CAA GAC AAG GGT CCT AAA ACA GGA TCA ATA 2566
Gly Ser Gln Lys Lys Ser Gln Asp Lys Gly Pro Lys Thr Gly Ser Ile
805 810 815

AAG AAG GAA AAA GCA GTA AAG CCA GAG GAA GGG CAG CTT GTG CAG CTG 2614
Lys Lys Glu Lys Ala Val Lys Pro Glu Glu Gly Gln Leu Val Gln Leu
820 825 830

GAA GAG AAA GGG CAG GGT TCA GTG CCC TGG TCA GTA TAT GGT GTC TAC 2662
Glu Glu Lys Gly Gln Gly Ser Val Pro Trp Ser Val Tyr Gly Val Tyr
835 840 845

ATC CAG GCT GCT GGG GGC CCC TTG GCA TTC CTG GTT ATT ATG GCC CTT 2710
Ile Gln Ala Ala Gly Gly Pro Leu Ala Phe Leu Val Ile Met Ala Leu
850 855 860 865

TTC ATG CTG AAT GTA GGC AGC ACC GCC TTC AGC ACC TGG TGG TTG AGT 2758
Phe Met Leu Asn Val Gly Ser Thr Ala Phe Ser Thr Trp Trp Leu Ser
870 875 880

TAC TGG ATC AAG CAA GGA AGC GGG AAC ACC ACT GTG ACT CGA GGG AAC 2806
Tyr Trp Ile Lys Gln Gly Ser Gly Asn Thr Thr Val Thr Arg Gly Asn
885 890 895

GAG ACC TCG GTG AGT GAC AGC ATG AAG GAC AAT CCT CAT ATG CAG TAC 2854
Glu Thr Ser Val Ser Asp Ser Met Lys Asp Asn Pro His Met Gln Tyr
900 905 910

TAT GCC AGC ATC TAC GCC CTC TCC ATG GCA GTC ATG CTG ATC CTG AAA 2902
Tyr Ala Ser Ile Tyr Ala Leu Ser Met Ala Val Met Leu Ile Leu Lys
915 920 925

GCC ATT CGA GGA GTT GTC TTT GTC AAG GGC ACG CTG CGA GCT TCC TCC 2950
Ala Ile Arg Gly Val Val Phe Val Lys Gly Thr Leu Arg Ala Ser Ser
930 935 940 945

CGG CTG CAT GAC GAG CTT TTC CGA AGG ATC CTT CGA AGC CCT ATG AAG 2998
Arg Leu His Asp Glu Leu Phe Arg Arg Ile Leu Arg Ser Pro Met Lys
950 955 960

TTT TTT GAC ACG ACC CCC ACA GGG AGG ATT CTC AAC AGG TTT TCC AAA 3046
Phe Phe Asp Thr Thr Pro Thr Gly Arg Ile Leu Asn Arg Phe Ser Lys
965 970 975

GAC ATG GAT GAA GTT GAC GTG CGG CTG CCG TTC CAG GCC GAG ATG TTC 3094
Asp Met Asp Glu Val Asp Val Arg Leu Pro Phe Gln Ala Glu Met Phe
980 985 990

ATC CAG AAC GTT ATC CTG GTG TTC TTC TGT GTG GGA ATG ATC GCA GGA 3142
Ile Gln Asn Val Ile Leu Val Phe Phe Cys Val Gly Met Ile Ala Gly
995 1000 1005

GTC TTC CCG TGG TTC CTT GTG GCA GTG GGG CCC CTT GTC ATC CTC TTT 3190
Val Phe Pro Trp Phe Leu Val Ala Val Gly Pro Leu Val Ile Leu Phe
1010 1015 1020 1025

TCA GTC CTG CAC ATT GTC TCC AGG GTC CTG ATT CGG GAG CTG AAG CGT 3238
Ser Val Leu His Ile Val Ser Arg Val Leu Ile Arg Glu Leu Lys Arg
1030 1035 1040

CTG GAC AAT ATC ACG CAG TCA CCT TTC CTC TCC CAC ATC ACG TCC AGC 3286
Leu Asp Asn Ile Thr Gln Ser Pro Phe Leu Ser His Ile Thr Ser Ser
1045 1050 1055

ATA CAG GGC CTT GCC ACC ATC CAC GCC TAC AAT AAA GGG CAG GAG TTT 3334
Ile Gln Gly Leu Ala Thr Ile His Ala Tyr Asn Lys Gly Gln Glu Phe
1060 1065 1070

CTG CAC AGA TAC CAG GAG CTG CTG GAT GAC AAC CAA GCT CCT TTT TTT 3382
Leu His Arg Tyr Gln Glu Leu Leu Asp Asp Asn Gln Ala Pro Phe Phe
1075 1080 1085

TTG TTT ACG TGT GCG ATG CGG TGG CTG GCT GTG CGG CTG GAC CTC ATC 3430
Leu Phe Thr Cys Ala Met Arg Trp Leu Ala Val Arg Leu Asp Leu Ile
1090 1095 1100 1105

AGC ATC GCC CTC ATC ACC ACC ACG GGG CTG ATG ATC GTT CTT ATG CAC 3478
Ser Ile Ala Leu Ile Thr Thr Thr Gly Leu Met Ile Val Leu Met His
1110 1115 1120

GGG CAG ATT CCC CCA GCC TAT GCG GGT CTC GCC ATC TCT TAT GCT GTC 3526
Gly Gln Ile Pro Pro Ala Tyr Ala Gly Leu Ala Ile Ser Tyr Ala Val
1125 1130 1135

CAG TTA ACG GGG CTG TTC CAG TTT ACG GTC AGA CTG GCA TCT GAG ACA 3574
Gln Leu Thr Gly Leu Phe Gln Phe Thr Val Arg Leu Ala Ser Glu Thr
1140 1145 1150

GAA GCT CGA TTC ACC TCG GTG GAG AGG ATC AAT CAC TAC ATT AAG ACT 3622
Glu Ala Arg Phe Thr Ser Val Glu Arg Ile Asn His Tyr Ile Lys Thr
1155 1160 1165

```
CTG TCC TTG GAA GCA CCT GCC AGA ATT AAG AAC AAG GCT CCC TCC CCT      3670
Leu Ser Leu Glu Ala Pro Ala Arg Ile Lys Asn Lys Ala Pro Ser Pro
1170                1175                1180                1185

GAC TGG CCC CAG GAG GGA GAG GTG ACC TTT GAG AAC GCA GAG ATG AGG      3718
Asp Trp Pro Gln Glu Gly Glu Val Thr Phe Glu Asn Ala Glu Met Arg
                1190                1195                1200

TAC CGA GAA AAC CTC CCT CTC GTC CTA AAG AAA GTA TCC TTC ACG ATC      3766
Tyr Arg Glu Asn Leu Pro Leu Val Leu Lys Lys Val Ser Phe Thr Ile
            1205                1210                1215

AAA CCT AAA GAG AAG ATT GGC ATT GTG GGG CGG ACA GGA TCA GGG AAG      3814
Lys Pro Lys Glu Lys Ile Gly Ile Val Gly Arg Thr Gly Ser Gly Lys
        1220                1225                1230

TCC TCG CTG GGG ATG GCC CTC TTC CGT CTG GTG GAG TTA TCT GGA GGC      3862
Ser Ser Leu Gly Met Ala Leu Phe Arg Leu Val Glu Leu Ser Gly Gly
    1235                1240                1245

TGC ATC AAG ATT GAT GGA GTG AGA ATC AGT GAT ATT GGC CTT GCC GAC      3910
Cys Ile Lys Ile Asp Gly Val Arg Ile Ser Asp Ile Gly Leu Ala Asp
1250                1255                1260                1265

CTC CGA AGC AAA CTC TCT ATC ATT CCT CAA GAG CCG GTG CTG TTC AGT      3958
Leu Arg Ser Lys Leu Ser Ile Ile Pro Gln Glu Pro Val Leu Phe Ser
                1270                1275                1280

GGC ACT GTC AGA TCA AAT TTG GAC CCC TTC AAC CAG TAC ACT GAA GAC      4006
Gly Thr Val Arg Ser Asn Leu Asp Pro Phe Asn Gln Tyr Thr Glu Asp
            1285                1290                1295

CAG ATT TGG GAT GCC CTG GAG AGG ACA CAC ATG AAA GAA TGT ATT GCT      4054
Gln Ile Trp Asp Ala Leu Glu Arg Thr His Met Lys Glu Cys Ile Ala
        1300                1305                1310

CAG CTA CCT CTG AAA CTT GAA TCT GAA GTG ATG GAG AAT GGG GAT AAC      4102
Gln Leu Pro Leu Lys Leu Glu Ser Glu Val Met Glu Asn Gly Asp Asn
    1315                1320                1325

TTC TCA GTG GGG GAA CGG CAG CTC TTG TGC ATA GCT AGA GCC CTG CTC      4150
Phe Ser Val Gly Glu Arg Gln Leu Leu Cys Ile Ala Arg Ala Leu Leu
1330                1335                1340                1345

CGC CAC TGT AAG ATT CTG ATT TTA GAT GAA GCC ACA GCT GCC ATG GAC      4198
Arg His Cys Lys Ile Leu Ile Leu Asp Glu Ala Thr Ala Ala Met Asp
                1350                1355                1360

ACA GAG ACA GAC TTA TTG ATT CAA GAG ACC ATC CGA GAA GCA TTT GCA      4246
Thr Glu Thr Asp Leu Leu Ile Gln Glu Thr Ile Arg Glu Ala Phe Ala
            1365                1370                1375

GAC TGT ACC ATG CTG ACC ATT GCC CAT CGC CTG CAC ACG GTT CTA GGC      4294
Asp Cys Thr Met Leu Thr Ile Ala His Arg Leu His Thr Val Leu Gly
        1380                1385                1390

TCC GAT AGG ATT ATG GTG CTG GCC CAG GGA CAG GTG GTG GAG TTT GAC      4342
Ser Asp Arg Ile Met Val Leu Ala Gln Gly Gln Val Val Glu Phe Asp
    1395                1400                1405
```

```
ACC CCA TCG GTC CTT CTG TCC AAC GAC AGT TCC CGA TTC TAT GCC ATG      4390
Thr Pro Ser Val Leu Leu Ser Asn Asp Ser Ser Arg Phe Tyr Ala Met
1410                1415                1420                1425

TTT GCT GCT GCA GAG AAC AAG GTC GCT GTC AAG GGC TGACTCCTCC           4436
Phe Ala Ala Ala Glu Asn Lys Val Ala Val Lys Gly
                1430                1435

CTGTTGACGA AGTCTCTTTT CTTTAGAGCA TTGCCATTCC CTGCCTGGGG CGGGCCCCTT    4496

CATCGCGTCC TCCTACCGAA ACCTTGCCTT TCTCGATTTT ATCTTTCGCA CAGCAGTTCC    4556

GGATTGGCTT GTGTGTTTCA CTTTTAGGGA GAGTCATATT TTGATTATTG TATTTATTCC    4616

ATATTCATGT AAACAAAATT TAGTTTTTGT TCTTAATTGC ACTCTAAAAG GTTCAGGGAA    4676

CCGTTATTAT AATTGTATCA GAGGCCTATA ATGAAGCTTT ATACGTGTAG CTATATCTAT    4736

ATATAATTCT GTACATAGCC TATATTTACA GTGAAAATGT AAGCTGTTTA TTTTATATTA    4796

AAATAAGCAC TGTGCTAAAA AAAAAAAAAA AAAAAAAAAA AGGGCGGCCG C             4847
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 1437 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
Met Lys Asp Ile Asp Ile Gly Lys Glu Tyr Ile Ile Pro Ser Pro Gly
 1               5                  10                  15

Tyr Arg Ser Val Arg Glu Arg Thr Ser Thr Ser Gly Thr His Arg Asp
            20                  25                  30

Arg Glu Asp Ser Lys Phe Arg Arg Thr Arg Pro Leu Glu Cys Gln Asp
        35                  40                  45

Ala Leu Glu Thr Ala Ala Arg Ala Glu Gly Leu Ser Leu Asp Ala Ser
    50                  55                  60

Met His Ser Gln Leu Arg Ile Leu Asp Glu Glu His Pro Lys Gly Lys
65                  70                  75                  80

Tyr His His Gly Leu Ser Ala Leu Lys Pro Ile Arg Thr Thr Cys Lys
                85                  90                  95

His Gln His Pro Val Asp Asn Ala Gly Leu Phe Ser Cys Met Thr Phe
            100                 105                 110

Ser Trp Leu Ser Ser Leu Ala Arg Val Ala His Lys Lys Gly Glu Leu
        115                 120                 125

Ser Met Glu Asp Val Trp Ser Leu Ser Lys His Glu Ser Ser Asp Val
    130                 135                 140
```

Asn Cys Arg Arg Leu Glu Arg Leu Trp Gln Glu Glu Leu Asn Glu Val
145 150 155 160

Gly Pro Asp Ala Ala Ser Leu Arg Arg Val Val Trp Ile Phe Cys Arg
165 170 175

Thr Arg Leu Ile Leu Ser Ile Val Cys Leu Met Ile Thr Gln Leu Ala
180 185 190

Gly Phe Ser Gly Pro Ala Phe Met Val Lys His Leu Leu Glu Tyr Thr
195 200 205

Gln Ala Thr Glu Ser Asn Leu Gln Tyr Ser Leu Leu Leu Val Leu Gly
210 215 220

Leu Leu Leu Thr Glu Ile Val Arg Ser Trp Ser Leu Ala Leu Thr Trp
225 230 235 240

Ala Leu Asn Tyr Arg Thr Gly Val Arg Leu Arg Gly Ala Ile Leu Thr
245 250 255

Met Ala Phe Lys Lys Ile Leu Lys Leu Lys Asn Ile Lys Glu Lys Ser
260 265 270

Leu Gly Glu Leu Ile Asn Ile Cys Ser Asn Asp Gly Gln Arg Met Phe
275 280 285

Glu Ala Ala Ala Val Gly Ser Leu Leu Ala Gly Gly Pro Val Val Ala
290 295 300

Ile Leu Gly Met Ile Tyr Asn Val Ile Ile Leu Gly Pro Thr Gly Phe
305 310 315 320

Leu Gly Ser Ala Val Phe Ile Leu Phe Tyr Pro Ala Met Met Phe Ala
325 330 335

Ser Arg Leu Thr Ala Tyr Phe Arg Arg Lys Cys Val Ala Ala Thr Asp
340 345 350

Glu Arg Val Gln Lys Met Asn Glu Val Leu Thr Tyr Ile Lys Phe Ile
355 360 365

Lys Met Tyr Ala Trp Val Lys Ala Phe Ser Gln Ser Val Gln Lys Ile
370 375 380

Arg Glu Glu Glu Arg Arg Ile Leu Glu Lys Ala Gly Tyr Phe Gln Ser
385 390 395 400

Ile Thr Val Gly Val Ala Pro Ile Val Val Val Ile Ala Ser Val Val
405 410 415

Thr Phe Ser Val His Met Thr Leu Gly Phe Asp Leu Thr Ala Ala Gln
420 425 430

Ala Phe Thr Val Val Thr Val Phe Asn Ser Met Thr Phe Ala Leu Lys
435 440 445

Val Thr Pro Phe Ser Val Lys Ser Leu Ser Glu Ala Ser Val Ala Val
450 455 460

Asp Arg Phe Lys Ser Leu Phe Leu Met Glu Glu Val His Met Ile Lys
465 470 475 480

Asn Lys Pro Ala Ser Pro His Ile Lys Ile Glu Met Lys Asn Ala Thr
485 490 495

Leu Ala Trp Asp Ser Ser His Ser Ser Ile Gln Asn Ser Pro Lys Leu
500 505 510

Thr Pro Lys Met Lys Lys Asp Lys Arg Ala Ser Arg Gly Lys Lys Glu
515 520 525

Lys Val Arg Gln Leu Gln Arg Thr Glu His Gln Ala Val Leu Ala Glu
530 535 540

Gln Lys Gly His Leu Leu Leu Asp Ser Asp Glu Arg Pro Ser Pro Glu
545 550 555 560

Glu Glu Glu Gly Lys His Ile His Leu Gly His Leu Arg Leu Gln Arg
565 570 575

Thr Leu His Ser Ile Asp Leu Glu Ile Gln Glu Gly Lys Leu Val Gly
580 585 590

Ile Cys Gly Ser Val Gly Ser Gly Lys Thr Ser Leu Ile Ser Ala Ile
595 600 605

Leu Gly Gln Met Thr Leu Leu Glu Gly Ser Ile Ala Ile Ser Gly Thr
610 615 620

Phe Ala Tyr Val Ala Gln Gln Ala Trp Ile Leu Asn Ala Thr Leu Arg
625 630 635 640

Asp Asn Ile Leu Phe Gly Lys Glu Tyr Asp Glu Glu Arg Tyr Asn Ser
645 650 655

Val Leu Asn Ser Cys Cys Leu Arg Pro Asp Leu Ala Ile Leu Pro Ser
660 665 670

Ser Asp Leu Thr Glu Ile Gly Glu Arg Gly Ala Asn Leu Ser Gly Gly
675 680 685

Gln Arg Gln Arg Ile Ser Leu Ala Arg Ala Leu Tyr Ser Asp Arg Ser
690 695 700

Ile Tyr Ile Leu Asp Asp Pro Leu Ser Ala Leu Asp Ala His Val Gly
705 710 715 720

Asn His Ile Phe Asn Ser Ala Ile Arg Lys His Leu Lys Ser Lys Thr
725 730 735

Val Leu Phe Val Thr His Gln Leu Gln Tyr Leu Val Asp Cys Asp Glu
740 745 750

Val Ile Phe Met Lys Glu Gly Cys Ile Thr Glu Arg Gly Thr His Glu
755 760 765

Glu Leu Met Asn Leu Asn Gly Asp Tyr Ala Thr Ile Phe Asn Asn Leu
770 775 780

Leu Leu Gly Glu Thr Pro Pro Val Glu Ile Asn Ser Lys Lys Glu Thr
785 790 795 800

Ser Gly Ser Gln Lys Lys Ser Gln Asp Lys Gly Pro Lys Thr Gly Ser
805 810 815

Ile Lys Lys Glu Lys Ala Val Lys Pro Glu Glu Gly Gln Leu Val Gln
820 825 830

Leu Glu Glu Lys Gly Gln Gly Ser Val Pro Trp Ser Val Tyr Gly Val
835 840 845

Tyr Ile Gln Ala Ala Gly Gly Pro Leu Ala Phe Leu Val Ile Met Ala
850 855 860

Leu Phe Met Leu Asn Val Gly Ser Thr Ala Phe Ser Thr Trp Trp Leu
865 870 875 880

Ser Tyr Trp Ile Lys Gln Gly Ser Gly Asn Thr Thr Val Thr Arg Gly
885 890 895

Asn Glu Thr Ser Val Ser Asp Ser Met Lys Asp Asn Pro His Met Gln
900 905 910

Tyr Tyr Ala Ser Ile Tyr Ala Leu Ser Met Ala Val Met Leu Ile Leu
915 920 925

Lys Ala Ile Arg Gly Val Val Phe Val Lys Gly Thr Leu Arg Ala Ser
930 935 940

Ser Arg Leu His Asp Glu Leu Phe Arg Arg Ile Leu Arg Ser Pro Met
945 950 955 960

Lys Phe Phe Asp Thr Thr Pro Thr Gly Arg Ile Leu Asn Arg Phe Ser
965 970 975

Lys Asp Met Asp Glu Val Asp Val Arg Leu Pro Phe Gln Ala Glu Met
980 985 990

Phe Ile Gln Asn Val Ile Leu Val Phe Phe Cys Val Gly Met Ile Ala
995 1000 1005

Gly Val Phe Pro Trp Phe Leu Val Ala Val Gly Pro Leu Val Ile Leu
1010 1015 1020

Phe Ser Val Leu His Ile Val Ser Arg Val Leu Ile Arg Glu Leu Lys
1025 1030 1035 1040

Arg Leu Asp Asn Ile Thr Gln Ser Pro Phe Leu Ser His Ile Thr Ser
1045 1050 1055

Ser Ile Gln Gly Leu Ala Thr Ile His Ala Tyr Asn Lys Gly Gln Glu
1060 1065 1070

Phe Leu His Arg Tyr Gln Glu Leu Leu Asp Asp Asn Gln Ala Pro Phe
1075 1080 1085

Phe Leu Phe Thr Cys Ala Met Arg Trp Leu Ala Val Arg Leu Asp Leu
1090 1095 1100

Ile Ser Ile Ala Leu Ile Thr Thr Thr Gly Leu Met Ile Val Leu Met
1105 1110 1115 1120

His Gly Gln Ile Pro Pro Ala Tyr Ala Gly Leu Ala Ile Ser Tyr Ala
1125 1130 1135

Val Gln Leu Thr Gly Leu Phe Gln Phe Thr Val Arg Leu Ala Ser Glu
1140 1145 1150

Thr Glu Ala Arg Phe Thr Ser Val Glu Arg Ile Asn His Tyr Ile Lys
1155 1160 1165

Thr Leu Ser Leu Glu Ala Pro Ala Arg Ile Lys Asn Lys Ala Pro Ser
1170 1175 1180

Pro Asp Trp Pro Gln Glu Gly Glu Val Thr Phe Glu Asn Ala Glu Met
1185 1190 1195 1200

Arg Tyr Arg Glu Asn Leu Pro Leu Val Leu Lys Lys Val Ser Phe Thr
1205 1210 1215

Ile Lys Pro Lys Glu Lys Ile Gly Ile Val Gly Arg Thr Gly Ser Gly
1220 1225 1230

Lys Ser Ser Leu Gly Met Ala Leu Phe Arg Leu Val Glu Leu Ser Gly
1235 1240 1245

Gly Cys Ile Lys Ile Asp Gly Val Arg Ile Ser Asp Ile Gly Leu Ala
1250 1255 1260

Asp Leu Arg Ser Lys Leu Ser Ile Ile Pro Gln Glu Pro Val Leu Phe
1265 1270 1275 1280

Ser Gly Thr Val Arg Ser Asn Leu Asp Pro Phe Asn Gln Tyr Thr Glu
1285 1290 1295

Asp Gln Ile Trp Asp Ala Leu Glu Arg Thr His Met Lys Glu Cys Ile
1300 1305 1310

Ala Gln Leu Pro Leu Lys Leu Glu Ser Glu Val Met Glu Asn Gly Asp
1315 1320 1325

Asn Phe Ser Val Gly Glu Arg Gln Leu Leu Cys Ile Ala Arg Ala Leu
1330 1335 1340

Leu Arg His Cys Lys Ile Leu Ile Leu Asp Glu Ala Thr Ala Ala Met
1345 1350 1355 1360

Asp Thr Glu Thr Asp Leu Leu Ile Gln Glu Thr Ile Arg Glu Ala Phe
1365 1370 1375

Ala Asp Cys Thr Met Leu Thr Ile Ala His Arg Leu His Thr Val Leu
1380 1385 1390

Gly Ser Asp Arg Ile Met Val Leu Ala Gln Gly Gln Val Val Glu Phe
1395 1400 1405

Asp Thr Pro Ser Val Leu Leu Ser Asn Asp Ser Ser Arg Phe Tyr Ala
1410 1415 1420

Met Phe Ala Ala Ala Glu Asn Lys Val Ala Val Lys Gly
1425   1430   1435

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 463 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GCGTCCGCCT AGAACGCAGA GATGAGGTAC CGAGAAAACC TCCCTCTCGT CCTAAAGAAA 60

GTATCCTTCA CGATCAAACC TAAAGAGAAG ATTGGCATTG TGGGGCGGAC AGGATCAGGG 120

AAGTCCTCGC TGGGGATGGC CCTCTTCCGT CTGGTGGAGT TATCTGGAGG CTGCATCAAG 180

ATTGATGGAG TGAGAATCAG TGATATTGGC CTTGCCGACC TCCGAAGCAA ACTCTCTATC 240

ATTCCTCAAG AGCCGGTGCT GTTCAGTGGC ACTGTCAGAT CAAATTTGGA CCCTTCAACC 300

AGTACACTGA AGACCAGATT TGGGATGCCC TGGAAAGGAC ACACATGAAA GAATGTATTG 360

CTCCAGCTAC CTCCTGAAAC TTGAATCCTG AATTTGATGG AGAAATGGGG AAATAACTTC 420

TCCAGTTGGG GGAAACGGCA CTCTTTGTTG CCATACCTAN ACC 463

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TGCTGGTTCT CTCCCTCACA CTTC 24

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CTCGGCTCGG GCTGCTGTTT CCAA 24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGGTGCTGGT GTTTGGAAGT AGTC 24

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GCCAGAGAAG AAAGCCACGA AAAA 24

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AGGCACACGA TGGACAGGAT GAGC 24

What is claimed is:

1. Nucleic acid, the sequence of which comprises SEQ ID No: 1.

2. Nucleic acid of clone fohd013a05m, ATCC Deposit No. 98409.

3. Nucleic acid, the sequence of which comprises a sequence complementary to SEQ ID No: 1 or to a unique fragment thereof.

4. Nucleic acid of claim 3 that is ribonucleic acid (RNA).

5. Nucleic acid, the sequence of which comprises a sequence at least 50% identical to SEQ ID No: 1.

6. Nucleic acid that hybridizes to SEQ ID No: 1 or to the complement thereof.

7. Nucleic acid of claim 6 that hybridizes under stringent conditions.

8. Nucleic acid, the sequence of which comprises a degenerate sequence variant of SEQ ID No:1.

9. Nucleic acid encoding a polypeptide, the amino acid sequence of which comprises SEQ ID No: 2.

10. Nucleic acid encoding a polypeptide, the amino acid sequence of which comprises a sequence sharing at least 75% sequence similarity with SEQ ID No: 2.

11. An oligonucleotide that hybridizes to a unique fragment of the nucleic acid of claim 10.

12. An oligonucleotide of claim 11 that hybridizes under stringent conditions.

13. An oligonucleotide of claim 11 that hybridizes under intracellular conditions.

14. An oligonucleotide of claim 11 comprising at least one modification in a nucleotide base, backbone sugar, phosphate or sugar-phosphate linkage.

15. A modified oligonucleotide of claim 14 comprising a peptide nucleic acid backbone.

16. A detectably labeled oligonucleotide of claim 11.

17. A biotinylated, radiolabeled or fluorophore-conjugated oligonucleotide of claim 16.

18. An oligonucleotide of claim 11 wherein said unique fragment is at least 9 nucleotides in length.

19. An oligonucleotide of claim 11 wherein said unique fragment is at least 15 nucleotides in length.

20. An oligonucleotide of claim 11 wherein said unique fragment is at least 21 nucleotides in length.

21. An oligonucleotide of claim 11 wherein said unique fragment is a locus comprising a 5' untranslated sequence, transcription initiation site, coding sequence, intron-exon boundary, polyadenylation site, or 3' untranslated sequence in the nucleic acid of claim 10.

22. An oligonucleotide, the sequence of which is selected from the group consisting of SEQ ID Nos: 4, 5, 6, 7 and 8.

23. An antisense vector comprising nucleic acid encoding an oligonucleotide of claim 11.

24. An antisense pharmaceutical composition comprising an oligonucleotide of claim 11 or a vector of claim 23 dispersed in a pharmaceutically acceptable vehicle.

25. An MRP-β polypeptide, the amino acid sequence of which comprises SEQ ID No: 2.

26. An MRP-β polypeptide, the amino acid sequence of which comprises a sequence sharing at least 75% sequence similarity with SEQ ID No: 2.

27. An epitope unique to the MRP-β polypeptide of claim 26.

28. An epitope of claim 27 that is displayed by a cell expressing an MRP-β gene.

29. An antibody that binds selectively to the epitope of claim 27.

30. An antigen-binding fragment of the antibody of claim 29.

31. A fusion polypeptide comprising an antigen-binding fragment of claim 30.

32. A fusion polypeptide of claim 31, further comprising a cytotoxic polypeptide, such that said fusion polypeptide stimulates cytolysis of a cell expressing an MRP-β gene.

33. A fusion polypeptide of claim 31, further comprising a chemoattractant, such that said fusion polypeptide stimulates destruction of a cell expressing an MRP-β gene by macrophages, killer T cells or cytotoxic T cells.

34. An expression vector comprising nucleic acid encoding the polypeptide of claim 26.

35. A cell transfected with an expression vector of claim 34.

36. A cell of claim 35 that is immortalized under cell culture conditions.

37. A cell of claim 36 that is of human origin.

38. A cell of claim 36 that is a unicellular organism.

39. A yeast cell of claim 38.

40. A cell of claim 35 that is a non-human mammalian embryonic blastocyst cell.

41. A non-human mammal produced by intrauterine implantation of a blastocyst comprising the cell of claim 40.

42. Progeny of the mammal of claim 41, said progeny characterized by germline integration of said nucleic acid encoding the MRP-β polypeptide of claim 25.

43. A null vector comprising nucleic acid encoding a non-expressible variant of the polypeptide of claim 26.

44. A cell transfected with the null vector of claim 43.

45. A cell of claim 44 that is a non-human mammalian embryonic blastocyst cell.

46. A non-human mammal produced by intrauterine implantation of a blastocyst comprising the cell of claim 45.

47. Progeny of the mammal of claim 46, said progeny characterized by germline integration of nucleic acid encoding a non-expressible variant of the polypeptide of claim 26.

48. A method of detecting a mutation in an MRP-β gene, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring a variant MRP-β gene, the sequence of which differs from SEQ ID No: 1 by at least one nucleotide substitution, insertion or deletion;

(b) releasing nucleic acids from said cellular tissue;

(c) combining, under hybridization conditions, said released nucleic acids with an oligonucleotide complementary to SEQ ID No: 1 or to a unique fragment thereof; and

(d) assaying said released nucleic acids for formation of a hybrid comprising said oligonucleotide, formation of which indicates that said mammal harbors at least one wild-type MRP-β gene allele, the sequence of which comprises SEQ ID No: 1.

49. A method of detecting expression of an MRP-β gene, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring cells expressing an MRP-β gene encoding a polypeptide of claim 25;

(b) releasing RNA from said cellular tissue;

(c) combining, under hybridization conditions, said released RNA with an oligonucleotide that hybridizes to the complement of SEQ ID No: 1 or a unique fragment thereof; and

(d) assaying said released RNA for formation of a hybrid comprising said oligonucleotide, formation of which indicates that cells of said tissue express said MRP-β gene.

50. The method of claim 48 or 49 wherein said cellular tissue is suspected of comprising transformed cells.

51. A method of characterizing multidrug-resistant phenotype of a transformed cell of mammalian origin, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring transformed cells;

(b) releasing RNA from said cellular tissue;

(c) combining, under hybridization conditions, said released RNA with an oligonucleotide that hybridizes to the complement of SEQ ID No: 1 or a unique fragment thereof; and

(d) assaying said released RNA for formation of a hybrid comprising said oligonucleotide, formation of which indicates presence of transformed cells having a multidrug-resistance phenotype.

52. The method of claim 48, 49 or 51 wherein said oligonucleotide comprises a peptide nucleic acid backbone.

53. A method of characterizing drug-resistant phenotype of a transformed cell of mammalian origin, comprising the steps of:

(a) obtaining cellular tissue from a mammal suspected of harboring transformed cells;

(b) contacting said tissue with an antibody of claim 29, under conditions such that, if cells of said tissue display said an epitope selectively bound by said antibody, an antibody-epitope complex forms; and,

(c) assaying said tissue for the presence of said complex, formation of which indicates presence of transformed cells having a drug-resistant phenotype in said mammal.

54. The method of claim 51 or 53 wherein said cellular tissue is of mammary, respiratory tract, urogenital tract, endocrine system or immune system origin.

55. The method of claim 54 wherein said cellular tissue is of mammary origin and comprises a breast biopsy sample.

56. The method of claim 54 wherein said cellular tissue is of respiratory tract origin and comprises a bronchoalveolar lavage sample.

57. The method of claim 54 wherein said cellular tissue is of urogenital tract origin and comprises an ovarian, uterine or cervical biopsy sample.

58. The method of claim 54 wherein said cellular tissue is of urogenital tract origin and comprises a prostate or testicular biopsy sample.

59. The method of claim 54 wherein said cellular tissue is of endocrine system origin and comprises a pancreatic biopsy sample.

60. The method of claim 54 wherein said cellular tissue is of immune system origin and comprises a spleen, bone marrow or lymph node biopsy sample.

61. A method of mitigating aberrant expression of an MRP-β gene, comprising the step of:

administering an antisense pharmaceutical composition of claim 24 to a mammal suffering from effects of said aberrant expression, under conditions sufficient to attenuate a phenotype associated therewith.

62. A method of mitigating aberrant activity of an MRP-β gene, comprising the step of:

administering an antisense pharmaceutical composition of claim 24 to a mammal suffering from effects of said aberrant activity, under conditions sufficient to attenuate a phenotype associated therewith.

63. A method of improving effectiveness of chemotherapy for a mammal afflicted with a multidrug-resistant tumor, comprising the steps of:

(a) administering a chemotherapeutic drug to said mammal; and,

(b) coadministering an antisense pharmaceutical composition of claim 24,

such that said antisense pharmaceutical composition mitigates resistance of said tumor to said chemotherapeutic drug.

64. A method of treating a mammal suffering from aberrant expression of an MRP-β gene, comprising the step of administering a fusion polypeptide of claim 32 or 33 to said mammal, in an amount effective for destroying cells aberrantly displaying an epitope of claim 27.

65. A method of treating a mammal suffering from aberrant activity of an MRP-β polypeptide, comprising the step of administering a fusion polypeptide of claim 32 or 33 to said mammal, in an amount effective for destroying cells aberrantly displaying an epitope of claim 27.

66. A method of treating a mammal afflicted with a multidrug-resistant tumor, comprising the step of administering a fusion polypeptide of claim 32 or 33 to said mammal, in an amount effective for destroying tumor cells displaying an epitope of claim 27.

67. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell of claim 35 with a candidate modulator of MRP-β;

(b) assaying the level of MRP-β gene expression in said cell, wherein a detectable fluctuation in said level indicates that said candidate is an MRP-β modulator.

68. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell of claim 35 with a candidate modulator of MRP-β;

(b) assaying the level of MRP-β polypeptide displayed by said cell, wherein a detectable fluctuation in said level indicates that said candidate is an MRP-β modulator.

69. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell of claim 35 with a substrate transported by MRP-β;

(b) contacting a cell of claim 35 with a candidate modulator of MRP-β;

(c) assaying the amount of said substrate exported by said cell, wherein a detectable fluctuation in said amount indicates that said candidate is an MRP-β modulator.

70. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell of claim 35 with a cytotoxin exported or sequestered by MRP-β;

(b) contacting a cell of claim 35 with a candidate modulator of MRP-β;

(c) assaying survival of said cell, a detectable fluctuation in which indicates that said candidate is an MRP-β modulator.

71. A method of identifying a modulator of MRP-β, comprising the steps of:

(a) contacting a cell of claim 35 with a cytotoxin exported by MRP-β;

(b) contacting a cell of claim 35 with a candidate modulator of MRP-β;

(c) assaying efflux of said cytotoxin from said cell, a fluctuation in which indicates that said candidate is an MRP-β modulator.

72. An MRP-β modulator identified by the method of claim 67, 68, 69, 70 or 71.

73. An MRP-β modulator of claim 72 that is an inhibitor.

74. An MRP-β modulator of claim 72 that is a small molecule.

75. A multidrug-resistance attenuating pharmaceutical composition comprising a modulator of claim 72 dispersed in a pharmaceutically acceptable vehicle.

76. A method of mitigating aberrant expression of an MRP-β gene, comprising the step of administering an MRP-β modulator to a mammal suffering from effects of said aberrant expression, under conditions sufficient to attenuate a phenotype associated therewith.

77. A method of treating a mammal suffering from aberrant activity of an MRP-β polypeptide, comprising the step of administering an MRP-β modulator to a mammal suffering from effects of said aberrant activity, under conditions sufficient to attentuate a phenotype associated therewith.

78. A method of improving effectiveness of chemotherapy for a mammal afflicted with a multidrug-resistant tumor, comprising the steps of:

(a) administering a chemotherapeutic drug to said mammal; and,

(b) coadministering a pharmaceutical composition of claim 75,

such that said composition mitigates resistance of said tumor to said chemotherapeutic drug.

79. The method of claim 78 wherein said tumor is of mammary, respiratory tract, urogenital tract, endocrine system of immune system origin.

Novel Multidrug Resistance-Associated Polypeptide

Abstract of the Disclosure

Compositions and methods are disclosed for improving the effectiveness of a chemotherapeutic regimen to eradicate multidrug-resistant transformed cells from the body of a mammal, preferably from the body of a human. The present disclosure capitalizes on the discovery of a novel multidrug-resistance associated protein (MRP), herein designated MRP-β. The disclosed compositions include MRP-β nucleic acids, including probes and antisense oligonucleotides, MRP-β polypeptides and antibodies, MRP-β expressing host cells, and non-human mammals transgenic or nullizygous for MRP-β. The disclosed methods include methods for attenuating aberrant MRP-β gene expression, protein production and/or protein function. In addition, methods are disclosed for identifying and using a modulator, such as an inhibitor, of MRP-β. Preferably, the modulator is a small molecule.

404PRHW5508/03.506588-1

```
          GGCTCATGCT  CGGGAGCGTG  GTTGAGCGGC  TGGCGCGGTT  GTCCTGGAGC  AGGGGCGCAG    60
                                                                       M   K   D     3
 GAATTCTGAT  GTGAAACTAA  CAGTCTGTGA  GCCCTGGAAC  CTCCACTCAG  AGAAG ATG AAG GAT   124

 I   D   I   G   K   E   Y   I   I   P   S   P   G   Y   R   S   V   R   E   R    23
ATC GAC ATA GGA AAA GAG TAT ATC ATC CCC AGT CCT GGG TAT AGA AGT GTG AGG GAG AGA   184

 T   S   T   S   G   T   H   R   D   R   E   D   S   K   F   R   R   T   R   P    43
ACC AGC ACT TCT GGG ACG CAC AGA GAC CGT GAA GAT TCC AAG TTC AGG AGA ACT CGA CCG   244

 L   E   C   Q   D   A   L   E   T   A   A   R   A   E   G   L   S   L   D   A    63
TTG GAA TGC CAA GAT GCC TTG GAA ACA GCA GCC CGA GCC GAG GGC CTC TCT CTT GAT GCC   304

 S   M   H   S   Q   L   R   I   L   D   E   E   H   P   K   G   K   Y   H   H    83
TCC ATG CAT TCT CAG CTC AGA ATC CTG GAT GAG GAG CAT CCC AAG GGA AAG TAC CAT CAT   364

 G   L   S   A   L   K   P   I   R   T   T   S   K   H   Q   H   P   V   D   N   103
GGC TTG AGT GCT CTG AAG CCC ATC CGG ACT ACT TCC AAA CAC CAG CAC CCA GTG GAC AAT   424

 A   G   L   F   S   C   M   T   F   S   W   L   S   S   L   A   R   V   A   H   123
GCT GGG CTT TTT TCC TGT ATG ACT TTT TCG TGG CTT TCT TCT CTG GCC CGT GTG GCC CAC   484

 K   K   G   E   L   S   M   E   D   V   W   S   L   S   K   H   E   S   S   D   143
AAG AAG GGG GAG CTC TCA ATG GAA GAC GTG TGG TCT CTG TCC AAG CAC GAG TCT TCT GAC   544

 V   N   C   R   R   L   E   R   L   W   Q   E   E   L   N   E   V   G   P   D   163
GTG AAC TGC AGA AGA CTA GAG AGA CTG TGG CAA GAA GAG CTG AAT GAA GTT GGG CCA GAC   604

 A   A   S   L   R   R   V   V   W   I   F   C   R   T   R   L   I   L   S   I   183
GCT GCT TCC CTG CGA AGG GTT GTG TGG ATC TTC TGC CGC ACC AGG CTC ATC CTG TCC ATC   664

 V   C   L   M   I   T   Q   L   A   G   F   S   G   P   A   F   M   V   K   H   203
GTG TGC CTG ATG ATC ACG CAG CTG GCT GGC TTC AGT GGA CCA GCC TTC ATG GTG AAA CAC   724
```

FIGURE 1A

L L E Y T Q A T E S N L Q Y S L L L V L 223
CTC TTG GAG TAT ACC CAG GCA ACA GAG TCT AAC CTG CAG TAC AGC TTG TTG TTA GTG CTG 784

G L L L T E I V R S W S L A L T W A L N 243
GGC CTC CTC CTG ACG GAA ATC GTG CGG TCT TGG TCG CTT GCA CTG ACT TGG GCA TTG AAT 844

Y R T G V R L R G A I L T M A F K K I L 263
TAC CGA ACC GGT GTC CGC TTG CGG GGG GCC ATC CTA ACC ATG GCA TTT AAG AAG ATC CTT 904

K L K N I K E K S L G E L I N I C S N D 283
AAG TTA AAG AAC ATT AAA GAG AAA TCC CTG GGT GAG CTC ATC AAC ATT TGC TCC AAC GAT 964

G Q R M F E A A A V G S L L A G G P V V 303
GGG CAG AGA ATG TTT GAG GCA GCA GCC GTT GGC AGC CTG CTG GCT GGA GGA CCC GTT GTT 1024

A I L G M I Y N V I I L G P T G F L G S 323
GCC ATC TTA GGC ATG ATT TAT AAT GTA ATT ATT CTG GGA CCA ACA GGC TTC CTG GGA TCA 1084

A V F I L F Y P A M M F A S R L T A Y F 343
GCT GTT TTT ATC CTC TTT TAC CCA GCA ATG ATG TTT GCA TCA CGG CTC ACA GCA TAT TTC 1144

R R K C V A A T D E R V Q K M N E V L T 363
AGG AGA AAA TGC GTG GCC GCC ACG GAT GAA CGT GTC CAG AAG ATG AAT GAA GTT CTT ACT 1204

Y I K F I K M Y A W V K A F S Q S V Q K 383
TAC ATT AAA TTT ATC AAA ATG TAT GCC TGG GTC AAA GCA TTT TCT CAG AGT GTT CAG AAA 1264

I R E E E R R I L E K A G Y F Q S I T V 403
ATC CGC GAG GAG GAG CGT CGG ATA TTG GAA AAA GCC GGG TAC TTC CAG AGC ATC ACT GTG 1324

G V A P I V V V I A S V V T F S V H M T 423
GGT GTG GCT CCC ATT GTG GTG GTG ATT GCC AGC GTG GTG ACC TTC TCT GTT CAT ATG ACC 1384

09526031 .091700

FIGURE 1B

```
 L   G   F   D   L   T   A   A   Q   A   F   T   V   V   T   V   F   N   S   M    443
CTG GGC TTC GAT CTG ACA GCA GCA CAG GCT TTC ACA GTG GTG ACA GTC TTC AAT TCC ATG  1444

 T   F   A   L   K   V   T   P   F   S   V   K   S   L   S   E   A   S   V   A    463
ACT TTT GCT TTG AAA GTA ACA CCG TTT TCA GTA AAG TCC CTC TCA GAA GCC TCA GTG GCT  1504

 V   D   R   F   K   S   L   F   L   M   E   E   V   H   M   I   K   N   K   P    483
GTT GAC AGA TTT AAG AGT TTG TTT CTA ATG GAA GAG GTT CAC ATG ATA AAG AAC AAA CCA  1564

 A   S   P   H   I   K   I   E   M   K   N   A   T   L   A   W   D   S   S   H    503
GCC AGT CCT CAC ATC AAG ATA GAG ATG AAA AAT GCC ACC TTG GCA TGG GAC TCC TCC CAC  1624

 S   S   I   Q   N   S   P   K   L   T   P   K   M   K   K   D   K   R   A   S    523
TCC AGT ATC CAG AAC TCG CCC AAG CTG ACC CCC AAA ATG AAA AAA GAC AAG AGG GCT TCC  1684

 R   G   K   K   E   K   V   R   Q   L   Q   R   T   E   H   Q   A   V   L   A    543
AGG GGC AAG AAA GAG AAG GTG AGG CAG CTG CAG CGC ACT GAG CAT CAG GCG GTG CTG GCA  1744

 E   Q   K   G   H   L   L   L   D   S   D   E   R   P   S   P   E   E   E   E    563
GAG CAG AAA GGC CAC CTC CTC CTG GAC AGT GAC GAG CGG CCC AGT CCC GAA GAG GAA GAA  1804

 G   K   H   I   H   L   G   H   L   R   L   Q   R   T   L   H   S   I   D   L    583
GGC AAG CAC ATC CAC CTG GGC CAC CTG CGC TTA CAG AGG ACA CTG CAC AGC ATC GAT CTG  1864

 E   I   Q   E   G   K   L   V   G   I   C   G   S   V   G   S   G   K   T   S    603
GAG ATC CAA GAG GGT AAA CTG GTT GGA ATC TGC GGC AGT GTG GGA AGT GGA AAA ACC TCT  1924

 L   I   S   A   I   L   G   Q   M   T   L   L   E   G   S   I   A   I   S   G    623
CTC ATT TCA GCC ATT TTA GGC CAG ATG ACG CTT CTA GAG GGC AGC ATT GCA ATC AGT GGA  1984

 T   F   A   Y   V   A   Q   Q   A   W   I   L   N   A   T   L   R   D   N   I    643
ACC TTC GCT TAT GTG GCC CAG CAG GCC TGG ATC CTC AAT GCT ACT CTG AGA GAC AAC ATC  2044
```

FIGURE 1C

```
 L   F   G   K   E   Y   D   E   E   R   Y   N   S   V   L   N   S   C   C   L    663
CTG TTT GGG AAG GAA TAT GAT GAA GAA AGA TAC AAC TCT GTG CTG AAC AGC TGC TGC CTG  2104

 R   P   D   L   A   I   L   P   S   S   D   L   T   E   I   G   E   R   G   A    683
AGG CCT GAC CTG GCC ATT CTT CCC AGC AGC GAC CTG ACG GAG ATT GGA GAG CGA GGA GCC  2164

 N   L   S   G   G   Q   R   Q   R   I   S   L   A   R   A   L   Y   S   D   R    703
AAC CTG AGC GGT GGG CAG CGC CAG AGG ATC AGC CTT GCC CGG GCC TTG TAT AGT GAC AGG  2224

 S   I   Y   I   L   D   D   P   L   S   A   L   D   A   H   V   G   N   H   I    723
AGC ATC TAC ATC CTG GAC GAC CCC CTC AGT GCC TTA GAT GCC CAT GTG GGC AAC CAC ATC  2284

 F   N   S   A   I   R   K   H   L   K   S   K   T   V   L   F   V   T   H   Q    743
TTC AAT AGT GCT ATC CGG AAA CAT CTC AAG TCC AAG ACA GTT CTG TTT GTT ACC CAC CAG  2344

 L   Q   Y   L   V   D   C   D   E   V   I   F   M   K   E   G   C   I   T   E    763
TTA CAG TAC CTG GTT GAC TGT GAT GAA GTG ATC TTC ATG AAA GAG GGC TGT ATT ACG GAA  2404

 R   G   T   H   E   E   L   M   N   L   N   G   D   Y   A   T   I   F   N   N    783
AGA GGC ACC CAT GAG GAA CTG ATG AAT TTA AAT GGT GAC TAT GCT ACC ATT TTT AAT AAC  2464

 L   L   L   G   E   T   P   P   V   E   I   N   S   K   K   E   T   S   G   S    803
CTG TTG CTG GGA GAG ACA CCG CCA GTT GAG ATC AAT TCA AAA AAG GAA ACC AGT GGT TCA  2524

 Q   K   K   S   Q   D   K   G   P   K   T   G   S   I   K   K   E   K   A   V    823
CAG AAG AAG TCA CAA GAC AAG GGT CCT AAA ACA GGA TCA ATA AAG AAG GAA AAA GCA GTA  2584

 K   P   E   E   G   Q   L   V   Q   L   E   E   K   G   Q   G   S   V   P   W    843
AAG CCA GAG GAA GGG CAG CTT GTG CAG CTG GAA GAG AAA GGG CAG GGT TCA GTG CCC TGG  2644

 S   V   Y   G   V   Y   I   Q   A   A   G   G   P   L   A   F   L   V   I   M    863
TCA GTA TAT GGT GTC TAC ATC CAG GCT GCT GGG GGC CCC TTG GCA TTC CTG GTT ATT ATG  2704

 A   L   F   M   L   N   V   G   S   T   A   F   S   T   W   W   L   S   Y   W    883
GCC CTT TTC ATG CTG AAT GTA GGC AGC ACC GCC TTC AGC ACC TGG TGG TTG AGT TAC TGG  2764
```

FIGURE 1D

```
 I   K   Q   G   S   G   N   T   T   V   T   R   G   N   E   T   S   V   S   D    903
ATC AAG CAA GGA AGC GGG AAC ACC ACT GTG ACT CGA GGG AAC GAG ACC TCG GTG AGT GAC  2824

 S   M   K   D   N   P   H   M   Q   Y   Y   A   S   I   Y   A   L   S   M   A    923
AGC ATG AAG GAC AAT CCT CAT ATG CAG TAC TAT GCC AGC ATC TAC GCC CTC TCC ATG GCA  2884

 V   M   L   I   L   K   A   I   R   G   V   V   F   V   K   G   T   L   R   A    943
GTC ATG CTG ATC CTG AAA GCC ATT CGA GGA GTT GTC TTT GTC AAG GGC ACG CTG CGA GCT  2944

 S   S   R   L   H   D   E   L   F   R   R   I   L   R   S   P   M   K   F   F    963
TCC TCC CGG CTG CAT GAC GAG CTT TTC CGA AGG ATC CTT CGA AGC CCT ATG AAG TTT TTT  3004

 D   T   T   P   T   G   R   I   L   N   R   F   S   K   D   M   D   E   V   D    983
GAC ACG ACC CCC ACA GGG AGG ATT CTC AAC AGG TTT TCC AAA GAC ATG GAT GAA GTT GAC  3064

 V   R   L   P   F   Q   A   E   M   F   I   Q   N   V   I   L   V   F   F   C   1003
GTG CGG CTG CCG TTC CAG GCC GAG ATG TTC ATC CAG AAC GTT ATC CTG GTG TTC TTC TGT  3124

 V   G   M   I   A   G   V   F   P   W   F   L   V   A   V   G   P   L   V   I   1023
GTG GGA ATG ATC GCA GGA GTC TTC CCG TGG TTC CTT GTG GCA GTG GGG CCC CTT GTC ATC  3184

 L   F   S   V   L   H   I   V   S   R   V   L   I   R   E   L   K   R   L   D   1043
CTC TTT TCA GTC CTG CAC ATT GTC TCC AGG GTC CTG ATT CGG GAG CTG AAG CGT CTG GAC  3244

 N   I   T   Q   S   P   F   L   S   H   I   T   S   S   I   Q   G   L   A   T   1063
AAT ATC ACG CAG TCA CCT TTC CTC TCC CAC ATC ACG TCC AGC ATA CAG GGC CTT GCC ACC  3304

 I   H   A   Y   N   K   G   Q   E   F   L   H   R   Y   Q   E   L   L   D   D   1083
ATC CAC GCC TAC AAT AAA GGG CAG GAG TTT CTG CAC AGA TAC CAG GAG CTG CTG GAT GAC  3364

 N   Q   A   P   F   F   L   F   T   C   A   M   R   W   L   A   V   R   L   D   1103
AAC CAA GCT CCT TTT TTT TTG TTT ACG TGT GCG ATG CGG TGG CTG GCT GTG CGG CTG GAC  3424
```

FIGURE 1E

```
 L   I   S   I   A   L   I   T   T   T   G   L   M   I   V   L   M   H   G   Q   1123
CTC ATC AGC ATC GCC CTC ATC ACC ACC ACG GGG CTG ATG ATC GTT CTT ATG CAC GGG CAG  3484

 I   P   P   A   Y   A   G   L   A   I   S   Y   A   V   Q   L   T   G   L   F   1143
ATT CCC CCA GCC TAT GCG GGT CTC GCC ATC TCT TAT GCT GTC CAG TTA ACG GGG CTG TTC  3544

 Q   F   T   V   R   L   A   S   E   T   E   A   R   F   T   S   V   E   R   I   1163
CAG TTT ACG GTC AGA CTG GCA TCT GAG ACA GAA GCT CGA TTC ACC TCG GTG GAG AGG ATC  3604

 N   H   Y   I   K   T   L   S   L   E   A   P   A   R   I   K   N   K   A   P   1183
AAT CAC TAC ATT AAG ACT CTG TCC TTG GAA GCA CCT GCC AGA ATT AAG AAC AAG GCT CCC  3664

 S   P   D   W   P   Q   E   G   E   V   T   F   E   N   A   E   M   R   Y   R   1203
TCC CCT GAC TGG CCC CAG GAG GGA GAG GTG ACC TTT GAG AAC GCA GAG ATG AGG TAC CGA  3724

 E   N   L   P   L   V   L   K   K   V   S   F   T   I   K   P   K   E   K   I   1223
GAA AAC CTC CCT CTC GTC CTA AAG AAA GTA TCC TTC ACG ATC AAA CCT AAA GAG AAG ATT  3784

 G   I   V   G   R   T   G   S   G   K   S   S   L   G   M   A   L   F   R   L   1243
GGC ATT GTG GGG CGG ACA GGA TCA GGG AAG TCC TCG CTG GGG ATG GCC CTC TTC CGT CTG  3844

 V   E   L   S   G   G   C   I   K   I   D   G   V   R   I   S   D   I   G   L   1263
GTG GAG TTA TCT GGA GGC TGC ATC AAG ATT GAT GGA GTG AGA ATC AGT GAT ATT GGC CTT  3904

 A   D   L   R   S   K   L   S   I   I   P   Q   E   P   V   L   F   S   G   T   1283
GCC GAC CTC CGA AGC AAA CTC TCT ATC ATT CCT CAA GAG CCG GTG CTG TTC AGT GGC ACT  3964

 V   R   S   N   L   D   P   F   N   Q   Y   T   E   D   Q   I   W   D   A   L   1303
GTC AGA TCA AAT TTG GAC CCC TTC AAC CAG TAC ACT GAA GAC CAG ATT TGG GAT GCC CTG  4024

 E   R   T   H   M   K   E   C   I   A   Q   L   P   L   K   L   E   S   E   V   1323
GAG AGG ACA CAC ATG AAA GAA TGT ATT GCT CAG CTA CCT CTG AAA CTT GAA TCT GAA GTG  4084
```

FIGURE 1F

M E N G D N F S V G E R Q L L C I A R A 1343
ATG GAG AAT GGG GAT AAC TTC TCA GTG GGG GAA CGG CAG CTC TTG TGC ATA GCT AGA GCC 4144

L L R H C K I L I L D E A T A A M D T E 1363
CTG CTC CGC CAC TGT AAG ATT CTG ATT TTA GAT GAA GCC ACA GCT GCC ATG GAC ACA GAG 4204

T D L L I Q E T I R E A F A D C T M L T 1383
ACA GAC TTA TTG ATT CAA GAG ACC ATC CGA GAA GCA TTT GCA GAC TGT ACC ATG CTG ACC 4264

I A H R L H T V L G S D R I M V L A Q G 1403
ATT GCC CAT CGC CTG CAC ACG GTT CTA GGC TCC GAT AGG ATT ATG GTG CTG GCC CAG GGA 4324

Q V V E F D T P S V L L S N D S S R F Y 1423
CAG GTG GTG GAG TTT GAC ACC CCA TCG GTC CTT CTG TCC AAC GAC AGT TCC CGA TTC TAT 4384

A M F A A A E N K V A V K G * 1437
GCC ATG TTT GCT GCT GCA GAG AAC AAG GTC GCT GTC AAG GGC TGA 4429

CTCCTCCCTGTTGACGAAGTCTCTTTTCTTTAGAGCATTGCCMYKGMMTKCCTGGGGCGGGCCCCTTCATCGCGTCCTC 4508

CTACCGAAACCTTGCCTTTCTCGATTTTATCTTTCGCACAGCAGTTCCGGATTGGCTTGTGTGTTTCACTTTTAGGGAG 4587

AGTCATATTTTGATTATTGTATTTATTCCATATTCATGTAAACAAAATTTAGTTTTTGTTCTTAATTGCACTCTAAAAG 4666

GTTCAGGGAACCGTTATTATAATTGTATCAGAGGCCTATAATGAAGCTTTATACGTGTAGCTATATCTATATATAATTC 4745

TGTACATAGCCTATATTTACAGTGAAAATGTAAGCTGTTTATTTTATATTAAAATAAGCACTGTGCTAAAAAAAAAAAA 4824

AAAAAAAAAAAAAGGGCGGCCGC 4847

FIGURE 1G

```
ALIGN calculates a global alignment of two sequences
 version 2.0uPlease cite: Myers and Miller, CABIOS (1989)
> SwissProt P33527 - MULTIDRUG RESISTANCE-ASSOCIA  1531 aa vs.
> MRP-H                                            1437 aa
scoring matrix: pam120.mat, gap penalties: -12/-4
30.9% identity;     Global alignment score: 1214

                10        20        30        40        50        60
inputs MALRGFCSADGSDPLWDWNVTWNTSNPDFTKCFQNTVLVWVPCFYLWACFPFYFLYLSRH

       ------------------------------------------------------------


                70        80        90       100       110       120
inputs DRGYIQMTPLNKTKTALGFLLWIVCWADLFYSFWERSRGIFLAPVFLVSPTLLGITTLLA

       ------------------------------------------------------------


               130       140       150       160       170
inputs TFLIQLERRKGVQSSGIMLTFWLVALVCALAILRSKIMTALKE-DAQVDLFRDITFYVYF
                                             : .  ::   .   .:..   .
       ----------------------------------MKDIDIGKEYIIPSPGYRSVRERTST
                                                    10        20

    180       190       200       210       220       230
inputs SLLLIQLVLSCFSDRSPLFSETIHDPNPCPES-SASFLSRITFWWITGLIVRG-YRQPLE
       :    .   : :    ::  .    . .. .:. : .   .   ...   .: :.. :
       SGTHRDREDSKFRRTRPLECQDALETAARAEGLSLDASMHSQLRILDEEHPKGKYHHGLS
         30        40        50        60        70        80

         240       250       260       270       280       290
inputs G-SDLWSLNKEDTSEQVVPVLV-KNWKKECAKTRKQPVKVVYSSKDP-AQPK-ESSKVDA
       .   ...   :  .  .  .  . ..  ..     . .:    :    :    :   .  . .: ::: :.
       ALKPIRTTCKHQHPVDNAGLFSCMTFSWLSSLARVAHKKGELSMEDVWSLSKHESSDVNC
         90       100       110       120       130       140

                300       310       320       330       340
inputs N--EEV--EALIVKSPQKEWNPSLFKVLYKTFGPYFLMSFFFKAIHDLMMFSGPQIL-KL
        : .  :.:   .:.   ..:: .:.         ...:.     : .:  :::: .. :
       RRLERLWQEELNEVGPD---AASLRRVVWIFCRTRLILSIVCLMITQLAGFSGPAFMVKH
        150       160            170       180       190       200

      350       360       370       380       390       400
inputs LIKFVNDTKAPDWQGYFYTVL-LFVTACLQTLVLHQYFHICFVSGMRIKTAVIGAVYRKA
       :. ....: ...  :    .   :: :..:. ...   :        . . .:.:.. :..   ...:
       LLEYTQAT-ESNLQYSLLLVLGLLLTEIVRSWSLALTWALNYRTGVRLRGAILTMAFKKI
           210       220       230       240       250       260

         410       420       430       440       450       460
inputs LVITNSARKSSTVGEIVNLMSVDAQRFMDLATYINMIWSAPLQVILALYLLWLNLGPSVL
       : .  :     :   ..::..:. : :.::   . :.   ... ..:. .::..      . :::. .
       LKLKN--IKEKSLGELINICSNDGQRMFEAAAVGSLLAGGFVVAILGMIYNVIILGPTGF
                270       280       290       300       310       320

         470       480       490       500       510       520
inputs AGVAVMVLMVPVNAVMAMKTKTYQVAHMKSKDNRIKLMNEILNGIKVLKLYAWELAFKDK
         : :: .:   :.       .   :    ..      . . :.:.. :::.:. :: .:.::    :: .
```

FIGURE 2A

```
         LGSAVFILFYPAMMFASRLTAYFRRKCVAATDERVQKMNEVLTYIKFIKMYAWVKAFSQS
                330       340       350       360       370       380

         530       540       550       560       570       580
inputs   VLAIRQEELKVLKKSAYLSAVGTFTWVCTPFLVALCTFAVYVTIDENNILDAQTAFVSLA
         :  ::.:: ..: :..:. .. . .    .   .... ::.: .:.. .  : :  ::. ..
         VQKIREEERRILEKAGYFQSITVGVAPIVVVIASVVTFSVHMTLGFD--LTAAQAFTVVT
                390       400       410       420        430

         590       600       610       620
inputs   LFNILRFPLNILPMVISSIVQASVSLKRLR-IFL-------------SHEELE-------
         .:: . :.:.. :  . :. .:::.. :.. .::              .:  .:
         VFNSMTFALKVTPFSVKSLSEASVAVDRFKSLFLMEEVHMIKNKPASPHIKIEMKNATLA
       440       450       460       470       480       490

               630          640              650
inputs   ----PDSIERRP--------VKDGGGTNSITVRN-------ATF----------TWARSD
             .::.  :         : ..  .  ::.         :..          . .:..
         WDSSHSSIQNSPKLTPKMKKDKRASRGKKEKVRQLQRTEHQAVLAEQKGHLLLDSDERPS
       500       510       520       530       540       550

                             660       670       680       690       700
inputs   PP-----------------TLNGITFSIPEGALVAVVGQVGCGKSSLLSALLAEMDKVEGH
         :                  ::..: . :.:: ::.. : :: ::.::.::.:..:  .::
         PEEEEGKHIHLGHLRLQRTLHSIDLEIQEGKLVGICGSVGSGKTSLISAILGQMTLLEGS
       560       570       580       590       600       610

               710       720       730       740       750       760
inputs   VAIKGSVAYVPQQAWIQNDSLRENILFGCQLEEPYYRSVIQACALLPDLEILPSGDRTEI
         .:: :. ::::.:::: :..::.::::: . .:  : ::...: : :::.::::.: :::
         IAISGTFAYVAQQAWILNATLRDNILFGKEYDEERYNSVLNSCCLRPDLAILPSSDLTEI
       620       630       640       650       660       670

               770       780       790       800       810       820
inputs   GEKGVNLSGGQKQRVSLARAVYSNADIYLFDDPLSAVDAHVGKHIFENVIGPKGMLKNKT
         ::.:.::::::.::.:::::.::. .::.::::::.:::::.:::...:   .   ::.::
         GERGANLSGGQRQRISLARALYSDRSIYILDDPLSALDAHVGNHIFNSAI--RKHLKSKT
       680       690       700       710       720        730

               830       840       850       860       870       880
inputs   RILVTHSMSYLPQVDVIIVMSGGKISEMGSYQELLARDGAFAEFLRTYASTEQEQDAEEN
          ..::: . :: . : .: :  : :.: :. .::.. .:..: ..              .:
         VLFVTHQLQYLVDCDEVIFMKEGCITERGTHEELMNLNGDYATIF-------------NN
          740       750       760       770       780

               890       900       910       920       930       940
inputs   GVTGVSGPGKEAKQMENGMLVTDSAGKQLQRQLSSSSSYSGDISRHHNSTAELQKAEAKK
          . : . :   . ..:        ..: : . :  .. .  :.: .          .:: .:
         LLLGETPPVEINSKKE--------TSGSQKKSQDKGPKT--GSIKK---------EKA-VKP
             790                800       810                  820

               950       960       970       980       990      1000
inputs   EETWKLMEADKAQTGQVKLSVYWDYMKAIGLFISFLSIF-LFMCNHVSALASNYWLSLWT
         ::   .  ..:.: : :  ::: :..: :  ..:: :  ::: :  :.  :. ::: :.
         EEGQLVQLEEKGQ-GSVPWSVYGVYIQAAGGPLAFLVIMALFMLNVGSTAFSTWWLSYWI
           830       840       850       860       870       880

                 1010      1020      1030          1040
```

FIGURE 2B

```
inputs DDPIVNGTQ---EHTKVRLSVYGALGISQGIAVFGYSMAV-----SIGGIL-------AS
       .   : :     . : :  :. ..  .     .... ::::     .: :..       ::
       KQGSGNTTVTRGNETSVSDSMKDNPHMQYYASIYALSMAVMLILKAIRGVVFVKGTLRAS
         890       900       910       920       930       940

        1050      1060      1070      1080      1090      1100
inputs RCLHVDLLHSILRSPMSFFERTPSGNLVNRFSKELDTVDSMIPEVIKMFMGSLFNVIGAC
        :: .:.. :::::: ::. ::.: ..::::::..: ::  .:     ::. ... :.  :
       SRLHDELFRRILRSPMKFFDTTPTGRILNRFSKDMDEVDVRLPFQAEMFIQNVILVF-FC
         950       960       970       980       990      1000

        1110      1120      1130      1140      1150      1160
inputs IVILLAT-PIAAIIIPPLGLIYFFVQRFYVASSRQLKRLESVSRSPVYSHFNETLLGVSV
       . .. .. :     . . :: ...   ..     .  :.:::::.....::  ::.. .. :...
       VGMIAGVFPWFLVAVGPLVILFSVLHIVSRVLIRELKRLDNITQSPPLSHITSSIQGLAT
        1010      1020      1030      1040      1050      1060

        1170      1180      1190      1200      1210      1220
inputs IRAFEEQERFIHQSDLKVDENQKAYYPSIVANRWLAVRLECVGNCIVLFAALFAVISRHS
       :.:..   . :.:. .   .:.:: ...   . : :::::::. ..   ..   ..:  :. .
       IHAYNKGQEFLHRYQELLDDNQAPFFLFTCAMRWLAVRLDLISIALITTTGLMIVLMHGQ
        1070      1080      1090      1100      1110      1120

        1230      1240      1250      1260      1270      1280
inputs LSAGLVGLSVSYSLQVTTYLNWLVRMSSEMETNIVAVERLKEYSETEK-EAPWQIQETAP
       .... .::.-::..:.:   ..   ::..:: :. ...:::.. :   :     ::: .:.. ::
       IPPAYAGLAISYAVQLTGLFQFTVRLASETEARFTSVERINHYIKTLSLEAPARIKNKAP
        1130      1140      1150      1160      1170      1180

        1290      1300      1310      1320      1330      1340
inputs PSSWPQVGRVEFRNYCLRYREDLDFVLRHINVTINGGEKVGIVGRTGAGKSSLTLGLFRI
       ...::: : : : :  .::::.: .::. .. ::.  ::.::::::::.::::: ..:::.
       SPDWPQEGEVTFENAEMRYRENLPLVLKKVSFTIKPKEKIGIVGRTGSGKSSLGMALFRL
        1190      1200      1210      1220      1230      1240

        1350      1360      1370      1380      1390      1400
inputs NESAEGEIIIDGINIAKIGLHDLRFKITIIPQDPVLFSGSLRMNLDPFSQYSDEEVWTSL
        : . : : :::. :. ::: ::: :..::::.::::::..: :::::.::.....: .:
       VELSGGCIKIDGVRISDIGLADLRSKLSIIPQEPVLFSGTVRSNLDPFNQYTEDQIWDAL
        1250      1260      1270      1280      1290      1300

        1410      1420      1430      1440      1450      1460
inputs ELAHLKDFVSALPDKLDHECAEGGENLSVGQRQLVCLARALLRKTKILVLDEATAAVDLE
       : .:.:. .. :: ::. :   :.:.:.:::.:::.:.::::::  :::.::::::::.: :
       ERTHMKECIAQLPLKLESEVMENGDNFSVGERQLLCIARALLRHCKILILDEATAAMDTE
        1310      1320      1330      1340      1350      1360

        1470      1480      1490      1500      1510      1520
inputs TDDLIQSTIRTQFEDCTVLTIAHRLNTIMDYTRVIVLDKGEIQEYGAPSDLL-QQRGLFY
       :: ::: :::   :.:::.:::::::::.:...   :..::..:.. :...:: :: .. . ::
       TDLLIQETIREAFADCTMLTIAHRLHTVLGSDRIMVLAQGQVVEFDTPSVLLSNDSSRFY
        1370      1380      1390      1400      1410      1420

           1530
inputs SM-AKDAGLV----
       .: : ... :
       AMFAAAENKVAVKG
        1430
```

FIGURE 2C

Customer Number: 000959

Attorney's Docket
Number MNI-056CP

Declaration, Petition and Power of Attorney
for Continuation-in-Part Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

NOVEL MULTIDRUG RESISTANCE-ASSOCIATED POLYPEPTIDE
the specification of which

(check one)

_ is attached hereto.

X was filed on April 16, 1998 as

Application Serial No. 09/061,400

and was amended on ____________.
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

This application in part discloses and claims subject matter disclosed in my earlier filed application(s), as follows:

_ Serial No.________, filed____________;
Serial No.________, filed____________, as to which I claim priority benefit under Title 35, United States Code, §119(e).

X Serial No. 08/843,459, filed April 16, 1997;
Serial No.________, filed____________, as to which I claim priority benefit under Title 35, United States Code, §120.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56, including all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of the continuation-in-part application.

AS TO PARENT APPLICATION:

As to the subject matter of this application which is common to said earlier application, I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to said earlier application, or in public use or on sale in the United States of America more than one year prior to said earlier application; that the common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of said earlier application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to said earlier application; and

As to applications for patents or inventor's certificate or PCT international application(s) designating at least one country other than the United States of America, on the common subject matter, filed in or designating any country foreign to the United States of America, prior to said earlier application by me or my legal representatives or assigns,

Check one:

X no such applications have been filed.

_ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID EARLIER U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			_ Yes No X
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _

ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO SAID EARLIER U.S. APPLICATION

AS TO THIS APPLICATION:

As to the subject matter of this application which is not common to said earlier application, I do not know and do not believe that the same was ever known or used in the United States of America before my or our invention thereof or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, or in public use or on sale in the United States of America more than one year prior to this application; that said non-common subject matter has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months prior to this application; and

As to applications for patents or inventor's certificate or PCT international application(s) designating at least one country other than the United States of America, on said non-common subject matter, filed in or designating any country foreign to the United States of America, prior to this application by me or my legal representatives or assigns,

Check one:

X no such applications have been filed.

_ such applications have been filed as follows

EARLIEST FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

Country	Application Number	Date of Filing (month,day,year)	Priority Claimed Under 35 USC 119
			_ Yes No X
			_ Yes No _
			_ Yes No _
			_ Yes No _
			_ Yes No _

ALL FOREIGN APPLICATION(S), IF ANY, FILED MORE THAN 12 MONTHS (6 MONTHS FOR DESIGN) PRIOR TO THIS U.S. APPLICATION

CLAIM FOR BENEFIT OF U.S. PROVISIONAL APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

CLAIM FOR BENEFIT OF U.S. PATENT APPLICATION(S)

I hereby claim the benefit under 35 U.S.C. §120 of any United States patent application(s) listed below.

08/843,459	April 16, 1997
(Application Serial No.)	(Filing Date)
(Application Serial No.)	(Filing Date)

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorneys and/or agents to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

W. Hugo Liepmann	Reg. No. 20,407	Lawrence E. Monks	Reg. No. 34,224
James E. Cockfield	Reg. No. 19,162	David A. Lane, Jr.	Reg. No. 39,261
Thomas V. Smurzynski	Reg. No. 24,798	Catherine J. Kara	Reg. No. 41,106
Ralph A. Loren	Reg. No. 29,325	Scott D. Rothenberger	Reg. No. 41,277
Thomas J. Engellenner	Reg. No. 28,711	Linda M. Chinn	Reg. No. 31,240
Giulio A. DeConti, Jr.	Reg. No. 31,503	Kevin J. Canning	Reg. No. 35,470
Ann Lamport Hammitte	Reg. No. 34,858	Faustino A. Lichauco	Reg. No. P41,942
Elizabeth A. Hanley	Reg. No. 33,505	C. Eric Schulman	Reg. No. P43,350
John V. Bianco	Reg. No. 36,748	Jeanne M. DiGiorgio	Reg. No. P41,710
Amy E. Mandragouras	Reg. No. 36,207	Megan E. Williams	Reg. No. P43,270
Anthony A. Laurentano	Reg. No. 38,220	Nicholas P. Triano III	Reg. No. 36,397
Jane E. Remillard	Reg. No. 38,872	Peter C. Lauro	Reg. No. 32,360
Jeremiah Lynch	Reg. No. 17,425		

all of: LAHIVE & COCKFIELD, LLP, 28 State Street, Boston, MA 02109

and to: Robert A. Millman Reg. No. 36,217 Jean M. Silveri Reg. No. 39,030
Mark F. Boshar Reg. No. 35,456 Theodore Allen Reg. No. P41,578

of: Millennium Pharmaceuticals, Inc., 238 Main Street, Cambridge, MA 02142-1017

Send Correspondence to Elizabeth A. Hanley, Esq. at **Customer Number 000959** whose address is:

Lahive & Cockfield, LLP, 28 State Street, Boston, MA 02109

Direct Telephone Calls to: (name and telephone number)

Elizabeth A. Hanley, Esq., (617) 227-7400

Wherefore I petition that letters patent be granted to me for the invention or discovery described and claimed in the attached specification and claims, and hereby subscribe my name to said specification and claims and to the foregoing declaration, power of attorney, and this petition.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor Andrew Shyjan	
Inventor's signature Andrew W. Shyjan	Date 8/31/98
Residence 25 Baker Road, Nahant, MA 01908	
Citizenship USA	
Post Office Address (if different) Same as above	